# Interaction of dietary protein and vitamin E supplementation on faecal egg counts, growth performance and haematological indices in lambs

By

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**Declaration**

I, Ayobami Dhikrullah Adeyemo, declare that this thesis has not been submitted to any university and the study is my original work conducted under the supervision of Prof. M. Chimonyo. All the help towards the success of this study and all the references contained herein have been duly credited.

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**List of abbreviations**

| | |
|---|---|
| ADG | average daily gain |
| BCS | body condition score |
| $CHL_3$ | cultured *Haemonchus* $L_3$ larvae |
| $CTL_3$ | cultured *Trichostrongylus* $L_3$ larvae |
| FEC | faecal egg counts |
| GIN | gastrointestinal nematode |
| Ht | haematocrit |
| IU | international unit |
| PCV | packed cell volume |
| Hb | haemoglobin |
| RBC | red blood count/erythrocyte |
| MCH | mean corpuscular haemoglobin |
| MCV | mean corpuscular volume |
| MCHC | mean corpuscular haemoglobin concentration |
| WBC | white blood count/leucocyte |
| PE | protein and vitamin E supplementation |
| PNE | protein and no vitamin E supplementation |
| NPE | no protein and vitamin E supplementation |
| NPNE | no protein and no vitamin E supplementation |

**Interaction of dietary protein and vitamin E supplementation on faecal egg counts, growth performance and haematological indices in lambs**

**By**

**A.D. ADEYEMO**

## Abstract

The broad objective of the study was to determine the interaction of dietary protein and vitamin E supplementation on FEC, growth performance and haematological indices in lambs. The objective was accomplished through a 2 × 2 factorial arrangement, with two factors (dietary protein at 150 g/day and vitamin E at 30 IU/kg BW/day). Dohne Merino lambs (n = 24) of both sexes were used. The lambs were assigned to each of the four treatment combinations (n = 6). Treatment 1 lambs received dietary protein and vitamin E (PE), Treatment 2 lambs received dietary protein and no vitamin E (PNE), and Treatment 3 lambs received dietary vitamin E and no protein (NPE) while those in Treatment 4 received neither dietary protein nor vitamin E supplementation (NPNE). Lambs were exposed to nematode contaminated *Pennisetum clandestinum* for eight weeks. Average daily gains, body condition scores, faecal egg counts, cultured *Haemonchus* larvae, cultured *Trichostrongylus* larvae, packed cell volume (PCV), erythrocytes, haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), FAMACHA scores, leucocytes, lymphocytes and eosinophils was monitored.

There was a significant effect of dietary protein supplementation and week on ADG and BCS. There was no interaction between dietary protein and vitamin E supplementation on ADG and BCS. There was an effect of dietary vitamin E supplementation on $CHL_3$ and $CTL_3$. There was a significant effect of dietary protein supplementation on $CTL_3$. Lambs that received PNE and

NPE treatment had lower ($P<0.05$) FEC, $CHL_3$ and $CTL_3$, whereas lambs that received PNE and NPNE, had the same ($P>0.05$) FEC, $CHL_3$ and $CTL_3$. There was an interaction between dietary protein and vitamin E supplementation on FEC, $CHL_3$ and $CTL_3$ in gastrointestinal nematode (GIN) naturally infected lambs.

The effect of dietary vitamin E supplementation influenced ($P<0.05$) MCH, MCHC, lymphocytes and low ($P<0.05$) eosinophil concentrations. Dietary protein supplementation increased ($P<0.05$) PCV, Hb, RBC and MCHC, and decreased ($P<0.05$) eosinophil concentrations and FAMACHA scores. Lamb that received PE and NPE treatment had the same ($P>0.05$) eosinophil concentrations than lambs that received PNE and NPNE treatment, which had higher ($P<0.05$) eosinophil concentrations. Lambs that received PE and NPE treatment had lower ($P<0.05$) FAMACHA scores than, lambs that received PNE and NPNE, which had higher ($P<0.05$) FAMACHA scores.

Also, treatment lambs that received PE and NPE had same but higher ($P>0.05$) MCV, than lambs that received PNE and NPNE, which had lower ($P<0.05$) MCV. Lambs that received PE and NPE had higher ($P<0.05$) MCHC than, lambs that received PNE and NPNE, which had the same ($P>0.05$) MCHC. Therefore, it can be concluded that the interaction of protein and vitamin E supplementation improved FEC, $CHL_3$, $CTL_3$, eosinophil concentrations, FAMACHA scores, MCV, and MCHC in lambs.

## Dedication

I dedicate this thesis to the Almighty creator, who gave me the strength, grace and opportunity to complete this work and to my sweet grandmother Alhaja Sikirat Ayoka and gorgeous wife Funmilola Adeyemo, and our daughter Tiwatope Faizah Adeyemo.

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## Table of Contents

## List of Tables

## List of Figures

## List of Appendices

# CHAPTER 1: General Introduction

## 1.1 Background

The damages caused by parasite infection on sheep production is recognized worldwide (Charlier *et al*., 2014). Farmers graze sheep on natural pasture. Climatic stability favours the survival and harbour gastrointestinal nematodes (GIN) on pasture (Marufu *et al*., 2008). Poor pasture and grazing management lead to the vulnerability of ruminant to nematodes. The matured parasite reside in the gut of host, causing associated pathophysiological discomfort to sheep (Van Wyk *et al*., 1999) and increase pasture contamination and rate of reinfection. Haemonchus, one of the major GIN parasites in sheep, sucks blood from the host intestine, which may cause haemorrhage in tissue. More so, *Trichostrongylus* infection results to diarrhoea. Treatment of GIN relies solely on anthelmintics. Levamisole, benzimidazole, avermectin, ivermectin, doramectin, moxidectin, closantel and milbemycins as common anthelmintics (Fleming *et al*., 2006). However, parasite activities on host may cause in-appetence and fatigue, hence, increase in lamb vulnerability to GIN infection.

The nutritional status of an animal may affect their response to GIN. Gastrointestinal parasite activity in the gut attack protein synthesis. Proteins are un-substitutable compounds which are broken down into essential amino acids needed for body metabolism. Dietary protein enhances growth and maintenance, especially in the intestine, muscle and tissue growth. Effective functioning of blood cells and antibody production depend on adequate protein consumption (Simone *et al*., 2005). Intestinal parasites disrupt and minimize the digestion, and utilization of dietary protein (Wallace *et al*., 1999). The damages caused by GIN to the blood cells and tissue may need to be restored since they are proteinaceous in nature. Supplementing dietary protein enhance blood cells and tissue performance with extra molecules lost during free radical production in a parasitic infection (Knox *et al*, 2003).

Vitamin E is a fat soluble antioxidant needed for optimum functioning of the muscular, circulatory, nervous, reproductive, and immune system (Larsen *et al*., 1988; Anugu *et al*., 2013). Vitamin E is absorbed in the gut of a host and motivates the immune cells, tissue and organ against foreign pathogens in an infected host. Han *et al*. (2006) reported that oral supplementation of dietary vitamin E enhanced cytokine function. Manipulation of the host nutrition, exploring dietary protein (Louvandini *et al*., 2006) and vitamin E supplementation (Martinez-Perez *et al*., 2014) may improve sheep performance against parasitism. The effect of dietary vitamin E supplementation on growth performance is yet to be documented.

Baker *et al*. (2002), reported a reduction in faecal egg counts (FEC), an increase in body weight gains and condition, and enhanced immune responsiveness in lambs supplied dietary protein and vitamin E supplementation (Anugu *et al*., 2013). The individual performance of dietary protein and vitamin E supplementation on parasitism has been overemphasized. However, there is a huge gap on the interaction between dietary protein and vitamin E supplementation on parasitism.

Globally, sheep production is mostly extensive. Therefore, the use of pasture or browses are irrevocable, and hence, acquiring GIN from such natural condition is undeniable. Increase in FEC, deteriorating growth rate, anaemia and decrease in haematological indices are indirect losses of GIN. Susceptibility of lambs to GIN, nematode resistance to anthelmintics and unavailability of a newer broad spectrum of anthelmintics, disturbed metabolism in digesting feed and utilization of nutrients, and sudden death are constraints. The interaction of dietary protein and vitamin E supplementation on lamb growth, faecal egg counts, and haematological indices is unknown.

## 1.2 Justification

Lambs are susceptible to infections and diseases, especially parasitism. The nutritional status of a host is challenged when infected with GIN and lamb performance is altered. Minimizing risks associated with nematode resistance to anthelmintics and safeguarding lamb health are the drive towards a nutritional therapeutic approach. The un-substitutable role of essential amino acids, which are derived from dietary protein and, the antioxidant function of fat soluble vitamin E cannot be overemphasized. Lambs maintained under an extensive grazing management, where climatic condition favours the morphological growth and survival of GIN, may require high nutritional demand.

The effectiveness of dietary protein supplementation to subside GIN consequences and enhance lamb performance may be dependent on dietary vitamin E supplementation. Investigating the interaction of dietary protein and vitamin E supplementation on FEC and fecundity, growth performance and haematological indices is exigent before recommendation. Interaction of dietary protein and vitamin E supplementation may boost immune responsiveness of sheep against parasitism. The study is expected to help mutton producers and nutritionists understand how the interaction of dietary protein and vitamin E supplementation may be used to fight parasitism. Findings from the study will assist extension agents with evidences to disseminate across to mutton producers.

## 1.3 Objectives

The broad objective was to determine the interaction of dietary protein and vitamin E supplementation on faecal egg counts, growth performance, and haematological indices.

The specific objectives were to:

1. Assess the interaction of dietary protein and vitamin E supplementation on FEC and growth performance in lambs; and

2. Determine the interaction of dietary protein and vitamin E supplementation on haematological indices in lambs.

## 1.4 Hypotheses

1. There is an interaction between dietary protein and vitamin E supplementation on FEC and growth performance in lambs.
2. There is an interaction between dietary protein and vitamin E supplementation on haematological indices in lambs.

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# CHAPTER 2: Literature review

## 2.1 Introduction

In the tropics and sub-tropics, the massive effect of GIN in sheep has led to the need for developing a sustainable nematode control strategies. Manipulation of host nutrition may be sustainable against GIN infestation (Khan *et al*., 2012; Anugu *et al*., 2013). The review discusses the possibilities of combating GIN through dietary protein and vitamin E supplementation in lambs.

## 2.2 Effects of gastrointestinal nematodes

Gastrointestinal worms inflict pain and discomfort to an infected sheep. Marufu *et al*. (2008) reported that rainfall, temperature and humidity contributes to nematode development. The excreted eggs in the faeces of an infected animal develops into $L_1$ and then moult into $L_2$, under a favourable weather condition. The $L_2$ survives on bacteria at this stage until it develops into $L_3$. The $L_3$ larvae then move up grass blades and become ready for ingestion. The $L_3$ is picked up when sheep are grazed on contaminated pasture and the $L_3$ nematode migrates down the intestine, then moult into $L_4$ (Figure 2.1) (Hale, 2006). At this stage, the health status of sheep is compromised (Van Wyk *et al*., 2004).

Coffey *et al*. (2007) reported that a complete nematode life cycle takes between 18 – 23 days and reproduction in nematodiosis involves both matured adult male and female worms. Young lambs are more susceptible to parasitic infection (Van Wyk *et al*., 2004). Parasitic activities may reduce feed intake and flow rate of the digesta.

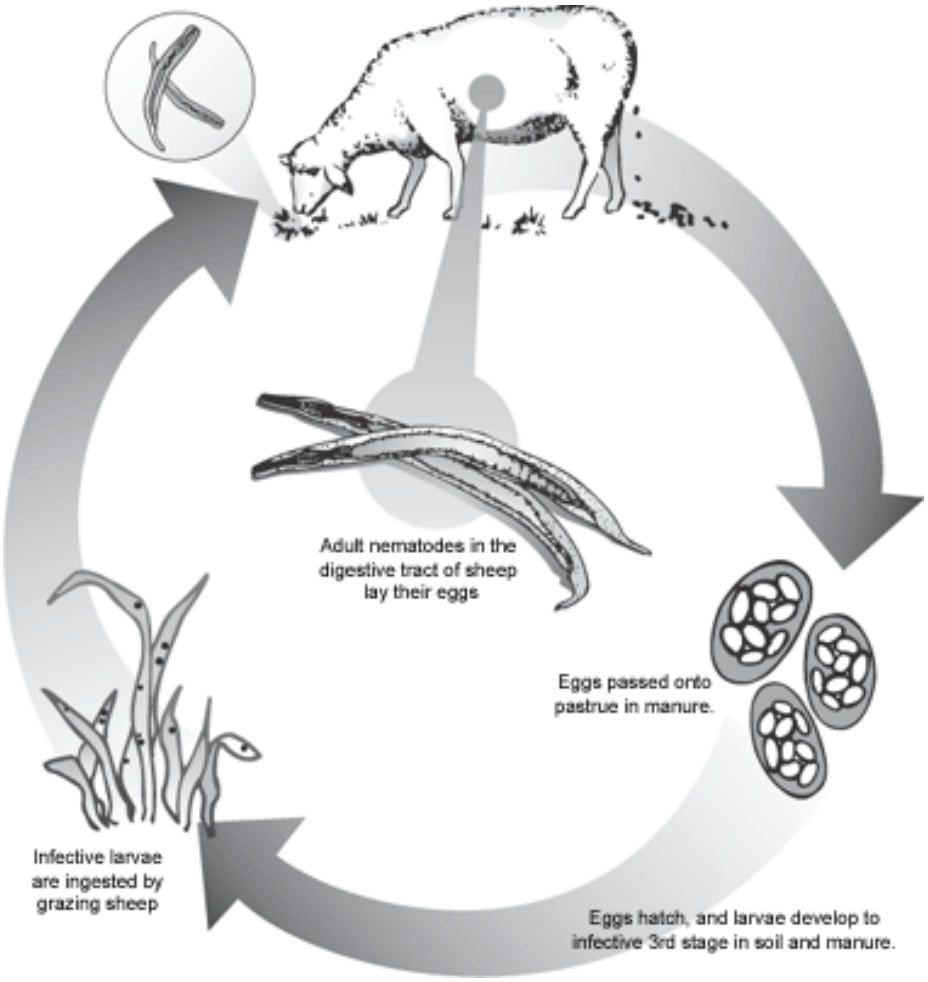


**Figure 2.1: Lifecycle of gastrointestinal nematode of sheep**

Source: Scheuerle (2009)

Submandibular oedema, fatigue, and diarrhoea are physical signs and symptoms of nematodiosis (Van Wyk *et al*., 2004). Hypoproteinaemia, malnutrition and in-absorption of nutrients in the gut, anaemia, anorexia, low milk production, reduced litter size, intestinal perforation and sudden death are graduated consequences (Van Wyk *et al*., 2004).

Haemonchus and *Trichostrongylus* reside in the abomasa mucosa. *Hemonchus* are known for their blood sucking characteristics, which may lead to a haemorrhage of the tissue (Valentine *et al*., 2007). *Haemonchus* and *Trichostrongylus* in the gut elevate the abomasa pH, through increased microbial processes (Simcock *et al*., 2006). These processes may be due to parasitic fecundity and GIN excreted product. Also, the matured GIN in the intestine alters gastrointestinal hormonal response, cholescytokin and gastrin. Therefore, an increase in hormone concentration (Fox, 1997).

Affected sheep may immediately show signs of parasitism, especially when they are nutritionally unstable. Sheep maintained under a field grazing pattern are more vulnerable and may be challenged with a mixed infection of different nematode genera. The impact of nematodiosis on the host is influenced by the extremity of the infection coupled with the immune status and the physiological structure of affected animal (Anugu *et al*., 2013). In Australia, the damages impacted by parasitism was estimated to be over $260 million per annum (Baker *et al*., 1990). High prevalence of parasitism was reported to be between 51 - 100 % in Germany (Moritz, 2005). A report shows that South Africa may have the highest percentage of *Haemonchus* infection in the world (Van Wyk *et al*., 1999). Measuring GIN fertility and epidemiology is essential since nematodes are threatening to sheep health and productivity.

## 2.3 Measures of nematodiosis

Several methods and strategies may be used to explain the impact of nematodiosis in sheep. These may include, body weight gains, faecal egg count, antibody production and concentrations, biochemical responses, haematological responses, and histopathological performance of sheep.

### 2.3.1 Body weight gain

Growth may be expressed as an increase in size or body weight gain and one criteria for assessing improvement in sheep. Increased body weight gains may be as a result of an efficient absorption and conversion of nutrients for body growth and maintenance (Haile *et al*., 2004). The inefficient absorption of nutrient into the gut for proper utilisation and metabolic processes affects body weight gains. Gastrointestinal parasite infection causes a huge loss in nitrogen through the faeces. Therefore, GIN activities induce a high loss of endogenous protein through faeces (Haile *et al*., 2004).

Elucidating body weight gains of an infected lamb is one vital tool in determining lamb growth. Poor nutrient availability in forage may affect sheep growth performance. Body weight gains of lambs naturally infected with mixed infection of *Haemonchus*, *Trichostrongylus*, *Trichuris* and *Moniezia* was monitored to evaluate the effect of nematodiosis in sheep (Louvandini *et al*., 2006). Gastrointestinal parasite activities in the rumen alter the efficient digestion of rumen microbe which function in body tissue growth. Induced pain, increased sheep vulnerability to GIN infection and decreased body weight gains are aggravated consequences. Body weight gains can be measured conventionally with the use of an electronic digital scale. Similarly, average daily weight gain of an animal is calculated by the difference between an initial weight

and the weight gain at the next weigh, then divided by the period between the initial and next body weight gain to arrive at the average gain.

$$\frac{\text{next weigh} - \text{Initial weight}}{\text{The period between the initial and next weigh}}$$

Salako and Ngere (2002), reported that mutton production is enhanced by the weight of mutton in some developed countries, whereby body weight gains of sheep is directly related to profitability in the mutton industry. There is scanty information on body weight gains of sheep naturally infected with a mixed GIN infection.

### 2.3.2 Body condition scores

Apart from the conventional measurement used in determining body weight gains in sheep, growth improvement can also be achieved through body condition scores. Condition scoring is a physical and indirect method of measuring fat deposition in livestock (Jefferies, 1961). The process is done by examining the amount of muscle and fat covering the lumber vertebrae in the loin region, rib cage and sternum of sheep. More so, caressing the spinous process around the lamb back carefully will help to effectively assess lamb body condition. Both hands can be used in assessing each spinous process to give an accurate condition of the ridges around the spine and assessing the transverse process to ascertain sharpness is necessary (Gerhart *et al*., 1996). In addition, assessing the rib cage intercostal spaces to confirm surrounding fat is of the essence. Body condition scores was used to evaluate the impact of *Haemonchus* and *Trichostrongylus* in experimentally infected goats (Mhomga *et al*., 2012).

### 2.3.3 Faecal egg counts

Nematode eggs are relatively small and oval in shape and may be in different sizes and colour (Table 2.1). However, nematode species share the same morphological growth and reproduction process. Faecal egg count is used in assessing the number of endoparasitic eggs present in the faeces of a host. The method may also be feasible in determining parasite burden and the species of worm present in an infected host (Van Wyk and Bath, 2002). Quantification of FEC before and after treatment may help to identify the effectiveness of treatment strategy. The number of eggs passed per gram (epg) of faeces may be influenced by host resistance ability, diarrhoea, nutritional status and the type of sexually matured parasites present in the intestine of the host (Love and Hutchinson, 2003). A nematode egg counts above 500 is considered high enough for a treatment intervention (Cole, 1986).

The increase in the bacteria content of the abomasum due to parasite activities causes a reduction in microbial abundance, resulting in pathogenic consequences, especially in the protein synthesis of infected host (Li *et al*., 2016). Faecal egg production in the gut of an infected animal elevates the abomasa pH. In addition, there is a strong relationship between GIN eggs production and elevated abomasum pH values in infected sheep (Simpson *et al*., 1997). The abomasa pH of infection with *Haemonchus* was higher in infected lambs than the non-infected group (Li *et al*., 2016). The pH of the non-infected group was 2.9, whereas the infected group had a 4.5 pH value (Li *et al*., 2016). Therefore, an elevated pH value may be due to a high faecal egg production in the abomasa mucosa of sheep infected with nematodiosis.

**Table 2.1: Nematode egg identification table**

| **Characteristics of nematode eggs** | ***Haemonchus*** | ***Trichostrongylus*** |
|---|---|---|
| Colour | Yellowish | Light brown |
| Length | 70 – 85 µm | 47 – 65 µm |
| Width | 44 µm | 25 – 26 µm |
| Comment | Relatively ovular in shape compared to other eggs | Circularly shaped and very small compared to other nematode eggs |

(Van Wyk *et al*., 2004)

### 2.3.4 Haematological indices

Packed cell volume (PCV) describes the volume percentage of erythrocytes (RBC) in the blood. Mean corpuscular volume (MCV) is a branch of RBC indices used in diagnosing the cause of anaemia in an infected lamb. Mean corpuscular haemoglobin (MCH) is the average mass of haemoglobin (Hb) in RBC. Mean corpuscular haemoglobin concentration (MCHC) is a branch of RBC indices which measures about average concentration of Hb in RBC. Mean corpuscular haemoglobin and MCHC function in diagnosing the type and the extremity of blood anaemia in a host. An alteration in the structure, mass or concentration of MCV, MCH and MCHC may indicate a mild or extreme anaemic condition in a host.

These haematological indices are responsible for diagnosis, prevention and expulsion of a viral, parasitic infection or disease. Erythrocytes are round shaped cells which protect the integrity and survival of the plasma membrane (Rodriguez *et al*., 2015). Haemoglobins and RBCs have a relationship function in oxygen absorption and transportation to cells and body tissue. A decrease in PCV indirectly affects RBC and Hb concentrations, since PCV percentage account for total RBC in an infected host and Hb concentrations may not be probably absorbed when RBC is low. Parasite manifestation may cause a reduction in the oxygen level in the plasma membrane due to their blood sucking characteristics from the gut (Martinez-Perez *et al.*, 2014). Microcytic anaemia is a depletion in the concentration of RBC, MCV, MCH and MCHC in parasited host.

The buccal cavity of *Haemonchus* attach to the abomasa wall of an infected sheep and causes severe pain and blood loss (Van Wyk *et al*., 2004). Urquhart (1996), reported that matured male and female *Haemonchus* in the abomasa mucosa sucks about 0.03 mL/worm/day. The blood loss in the abomasa mucosa of sheep resulted into body weight loss, a decrease in wool

growth, and reduced feed intake (Hayat *et al*., 1996). A report on Hb concentrations in infected sheep revealed that GIN induced a gradual reduction ($P<0.05$) in Hb concentrations when sheep are exposed to an experimental GIN infection (Bordoloi *et al*., 2012). Similarly, PCV declined ($P<0.05$) in sheep experimentally infected with nematodiosis and a reduction ($P<0.01$) in RBCs was observed in the same group of sheep (Bordoloi *et al*., 2012). Also, iron deficiency, malnutrition, in-absorption of nutrients and reduced haemoglobin concentrations may also lead to blood loss in the gut (Rodriguez *et al*., 2015).

Leucocytes combat infection and foreign pathogens which may be dangerous to the health of an animal (Meeusen and Balic, 2001). Hypoproteinaemia may lead to severe anaemia, reduced immunity and increase vulnerability to GIN infection. Eosinophil concentration are usually low in the blood. An increase in blood eosinophil concentrations may be associated with a synthetic reaction, inflammatory disorder, or parasitism (Rodriguez *et al*., 2015). Lymphocytes function through increased cytotoxic granules, enhanced antibody, and T helper cells necessary for immune activation against foreign pathogen. A prolonged infection may lead to lymphocytopenia or lymphocytosis and dangerous to lamb health status. The haematological response of GIN infected sheep need to be monitored, since GIN affects effective blood cell function.

### 2.3.5 FAMACHA

The FAMACHA technique was developed in Onderstepoort Veterinary Institute (OVI), South Africa (Van Wyk *et al.,* 1996). The technique may be used to identify a sick animal within a flock. The procedure was established for small resource farmers in assisting them to adequately and cheaply assess sheep health without the use of laboratory equipments or diagnosis (Van Wyk and Bath, 2002). Most sheep producers adopt the use of the FAMACHA technique to

identify the most vulnerable animal among flock (Kaplan, 2004). Examining the lower eyelid for possible anaemia may be necessary in an infected animal. Based on the colour of the eyelid, an immediate treatment can be given to affected animal (Mahieu *et al.*, 2007). The colour of the mucous membrane may be scored between 1 and 5 to ascertain the severity of the parasitic infection (Red – White). Lambs with a mild or severe signs of anaemia may be due to malnutrition or hyperproteinaemia. The FAMACHA technique may be used in diagnoses and a good indication of an anaemic condition which may challenge sheep health status (Rodriguez *et al*., 2015).

### 2.3.6 Histopathology

Assessing the intestinal cells and tissue may be useful in qualification and quantification of GIN damages. The histological assessment of the abomasa mucosa of a host explains the severity of GIN infection and worm burden in sheep. Examination of the intestinal tissue and organ, and presence of lymphoid follicles and eosinophils at the site of infection may also explain GIN impact in infected host (Martinez-Perez *et al*., 2014). The inflammatory activity of GIN in the gastrointestinal tract leads to the activation of the mast cell responses, which engage the release of histamine and other associated inflammatory receptors (Voehringer, 2013). The mast cell activities are characterized by their allergic responsiveness at the site of infection. Mast cells may increase during a parasitic infection. Assessing the histopathological impact of GIN on intestinal organ and tissue is warranted in an infected host (De wolf *et al*., 2014).

### 2.3.7 Immunology

Matured sheep are more immune to infection and harbour fewer adult worms. However, consistent exposure to some level of parasitism may be required to maintain their immunity

(McKenna, 1981). The main function of B lymphocytes is in the production of antibodies against foreign pathogen. The fusion of pathogens to B lymphocytes coupled with signalling from T helper cells activates lymphocytes to multiply and diffuse into the blood, which then produces a large amount of antibodies (immunoglobulins) in the blood which then fight parasitism (McRae *et al*., 2014).

Few numbers of antibodies are affected by GIN, immunoglobulin A (IgA), immunoglobulin G1 (IgG1) and immunoglobulin E (IgE). Immunoglobulin A (IgA) is commonly found at the site of a GIN infection. An increased production of IgA at the site of infection is related to Teladorsagia resistance and GIN fecundity (McRae *et al*., 2014).

### 2.3.8 Biochemical response

Serum enzymes are made up of proteins and the activities of GIN may elevate the concentration of serum enzymes. Total protein (TP), globulin (Glob), serum protein (SP), serum albumin (SAL), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are used in parasitic diagnosis and prognosis (Bordoloi *et al*., 2012). Total protein, Glob, SP and SAL are essential serum protein involved in osmotic regulation of the plasma in transporting lipids and steroid hormones. Lipids enhance the immune function of the cell. Serum enzymes are used to monitor a change in the physiological pattern of the blood or liver. The increase in serum enzyme levels are signs of perilous activities in organ and tissues. An altered membrane permeability may increase serum enzyme levels. Alanine aminotransferase and AST varied significantly after a complete *Haemonchus* life cycle in host body (Bordoloi *et al*., 2012).

Serum protein and serum albumin level significantly decreased in an experimental infection of GIN. A fall in SP and SAL normal threshold is known as hypoproteinaemia and hypoalbuminaemia (Bordoloi *et al*., 2012). Oedema in sheep is a physiological sign and symptoms of hypoproteinaemia and hypoalbuminaemia. Similarly, elevated ALP, ALT and AST concentrations in sheep may be attributed to GIN activities in the host abomasa mucosa (Bordoloi *et al*., 2012).

## 2.4 Nematode control

Since the economic importance of GIN compromises lambs performance and production, there is a need for a method of controlling nematodiosis. Pasture management (Van Wyk *et al*., 1999), efficacious anthelmintic product (Kohler, 2001) and nutritional therapy (Louvandini *et al*., 2006; Khan *et al*., 2012; Martinez-Perez *et al*., 2014) are used for controlling GIN.

### 2.4.1 Pasture management

Over the years, pasture management has been one of the methods of controlling GIN which may reduce the rate of re-infection and in turn create a safe pasture for grazing (Van Wyk *et al*., 1999). Coincidentally, weather instability of some areas rendered pasture management void. Controlling stocking density, rotational grazing, and segregation of age differences in more intensified production system are strategies used in the control of GIN infection. However, the control strategy require adequate knowledge into the epidemiology of nematode genera, seasonal availability, climate change, flock structure, and capital intensive.

### 2.4.2 Use of anthelmintics

Parasite control depend on the effectiveness of anthelmintic. Ivermectin, doramectin, moxidectin and closantel are drugs used against the premature and mature stages of GIN (Kohler, 2001). Most anthelmintics are used in improving protein kinases in regard to GIN control. Benzimidazole effect is by binding to B-tubulin in the formation of microtubules (Kohler, 2001). Avermectins binds with the protein glutamate and gamma-amino butyric acid, which then halt or eliminate the parasite from host (Prichard, 2001). Coincidentally, anthelmintics resistance by nematode has been reported (Fleming *et al*., 2006).

In addition, the whole class of anthelmintics, levamisole, benzimidazole, avermectins, ivermectin, doramectin, moxidectin, closantel and milbemycins are affected by nematode resistance (Fleming *et al*., 2006). Resistance can occur in various forms, which may involve a change in the molecular target, whereby the synthetic drug fails to recognize the targeted parasite, thus, inefficient (Van Wyk and Malan, 1988). Also, a change in operational activity of the activator, evacuating the drug from its target, or avert it activation. Furthermore, a change in the way of diffusion of the active agent, stopping the active agent from accessing its spot of operation; and multiplication of targeted genes which in turn overpowers the drug's activity.

Benzimidazole resistance is related to mutations in β-tubulin genes, which intercept the active agent fusion (Prichard, 2001). Similarly, avermectin and milbemycin resistance is related to a change in glutamate-gated chloride channelling (GluCl) and or, gamma-aminobutyric acid (GABA), an overexpression of P-glycoproteins and levamisole which mutation is associated with a change in nicotinic acetylcholine receptors. Anthelmintic resistance of susceptible lamb maintained under natural condition is not well documented, or rather outdated as many of these

reports were done over a decade or more (Table 2.2) (Watson *et al*., 1996; Van Wyk and Malan, 1999; Wolstenholme *et al*., 2004; Kaplan, 2006; Fleming *et al*., 2006).

Notably, anthelmintic treatment strategy is considered a forceful host immune responsiveness to parasitism. The most sustainable host defence response against parasitism is reported to be an acquired immunity (Stear *et al*., 1996). However, this is developed over time in relation to GIN challenge and determined by age and the nutritional status of such animal (Houdijk *et al*., 2005).

### 2.4.3 Nutrition

Nematodiosis may be considered a nutritional parasitism since parasite activities induce in-appetence, inefficient digestion and removal of nutrients needed for tissue maintenance and restoration (Hoste *et al*., 1997).

Manipulation of host nutrition may supply the nutrient required to improve immunity against parasitism or preserve the tissue or haematological responses and enhance productivity, despite the existence of parasitism. It has been reported that GIN pathogenesis affects dietary protein synthesis, rather than other nutrient components, including dietary energy (Bown *et al*., 1991). Proteins are complex compounds which play a critical role in body metabolism. Protein consists of hundreds of thousands of smaller units, namely amino acids, which are bonded together in chains.

**Table 2.2 Anthelmintic used in gastrointestinal nematode (GIN) control in sheep**

| ANTHELMINTIC | TARGETED HOST | YEAR OF INVENTION | FIRST RESISTANCE REPORT | REFERENCES |
|---|---|---|---|---|
| Thiabendazole | Sheep | 1961 | 1964 | (Conway 1964) |
| Levamisole | Sheep | 1970 | 1979 | (Sangster *et al*., 1979) |
| Ivermectin | Sheep | 1981 | 1988 | (Van Wyk and Malan, 1988) |
| Moxidectin | Sheep | 1991 | 1995 | (Watson *et al*., 1996) |

They contribute to various functions, such as, the fusion of antibodies to protect the body against foreign pathogens (Simone *et al*., 2005); enhancement of enzymes chemical processes in the cells and giving support with the formation of new molecules, translating the genetic information in DNA. In addition, protein exists in signalling between cells, organs and tissues through hormones; they also provide supports for cell structures on a large scale and regulate atoms and small molecules through body cells.

Vitamin E is a fat soluble antioxidant and a critical nutrient in animal health, inclusively human. The nutrient has proven essential against free radical injury (Smith and Bryant, 1989*a*) and strengthening the immune system, and mitigating mortality in pregnant ewe (Anugu *et al*., 2013). Vitamin E are closely related compounds of plant origin and occur in two forms, tocopherols and tocotrienols. Han and Meydani (2000), reported the most active ingredient form of vitamin E in nature to be α-tocopherol.

The main site of absorption in the body is the intestinal compartment and it is either dispensed as esters or alcohol. Damages caused by GIN in the body of the host are associated with chemical changes in the cells, known as lipid peroxidation (Rehim *et al*., 2003). Intestinal parasites decreases the amino acids needful for optimum functioning of the intestinal cells and tissue (Wallace *et al*., 1999). The lining tissues in the intestine are challenged with the needful nutrients essential for adequate and efficient growth. Body tissues and immune system seems to be competing for the little amino acids derived from grazed pasture in parasitized sheep. The larvae and matured GIN inflicts severe damage to and sloughing of the abomasa and intestinal wall, extracellular substances, leakage of plasma and increase in mucus development (Wallace *et al*., 1999). The damages impacted by GIN to tissue and cell need to be restored since they are proteinaceous in nature.

Similarly, the host responses to GIN infection is by stimulating effector cells, organs and tissues which fight foreign pathogen in the body of the host (Larsen *et al*., 1988). Vitamin E, an antioxidant which multiplies T cells through increased cellular components of cytokine interleukin 2 (IL-2) (Meydani *et al*., 1990). Type helper, Th1 and Th2 of an infected animal may be moved to Th1 type response in the course of vitamin E supplementation (Han, 2006). Oral supplementation of vitamin E has proven feasible increasing the production of IL-2, a vital Th cytokine (Han, 2006). Since naturally manufactured antioxidant are not sufficient to suppress GIN processes, it's important to provide infected hosts with extra antioxidant to regulate the emergence need. Hence, strengthening components of the immune system in ruminant through improved antibody performance against foreign pathogens (Politis *et al*., 1995).

Dietary protein supplementation increases body weight gain of Merino lamb at different inclusion levels (Deng *et al*., 2001). However, Martinez-Perez *et al*. (2014) reported low body weight gain in liver fluke parasitic lamb supplied with dietary vitamin E. More so, lamb body weight gains were not established in recent studies on GIN (MacGlafin *et al*., 2011; De wolf *et al*., 2014). Mhomga *et al*. (2012), reported a significant effect of high dietary protein supplementation on BCS in goat naturally infected with a mixed infection of *Haemonchus* and *Trichostrongylus*. Similarly, Khan *et al*. (2012), reported that dietary protein supplementation significantly increased BCS in experimentally infected lambs. There is no information on the effect of dietary vitamin E supplementation on BCS in lambs.

Deng *et al*. (2001) explained that young merino sheep supplied a diet that contained cottonseed meal at different levels had reduced FEC. Results on the effect of dietary vitamin E supplementation on FEC was not significant (MacGlafin *et al*., 2011; De wolf *et al*., 2014).

However, dietary vitamin E supplementation had a 49 % reduction on FEC (De wolf *et al*., 2014). Increased dietary vitamin E supplementation at 10 IU/kg BW/day or 5.3 IU/kg BW/day reduced FEC fecundity of experimentally infected lambs over time (Figure 2.2).

Packed cell volume (PCV) and haemoglobin (Hb) increased in GIN infected lambs supplied high dietary protein supplement (Khan *et al*., 2012). Similarly, there was an influence of high dietary protein supplementation on eosinophil concentration in lambs (Khan *et al*., 2012). Dietary vitamin E supplementation had no effect on PCV (MacGlafin *et al*., 2011).

Martinez-Perez *et al*. (2014), reported that dietary vitamin E supplementation on sheep experimentally infected with Fasciliosis, a liver fluke kind of parasitism, may improve certain haematological performance like, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). However, dietary vitamin E supplementation may not improve leucocyte (WBC), lymphocytes and erythrocytes (RBC) performance. Recent studies on dietary protein supplementation did not investigate the nutrient effect on WBC, RBC, MCH, MCHC (Louvandini *et al*., 2006; Khan *et al*., 2012).

The level of dietary protein and vitamin E in a diet, forage or feed influences the ability of infected host to fight parasitism (Han, 2006; Louvandini *et al*., 2006; Martinez-Perez *et al*., 2014). Successful individual performance of dietary protein (Deng *et al*., 2001; Louvandini *et al*., 2006; Khan *et al*., 2012) and vitamin E supplementation (De wolf *et al*., 2011; Anugu *et al*., 2013; Martinez-Perez *et al*., 2014) improved host performance against parasitic infections, especially *Haemonchus* and *Trichostrongylus*.

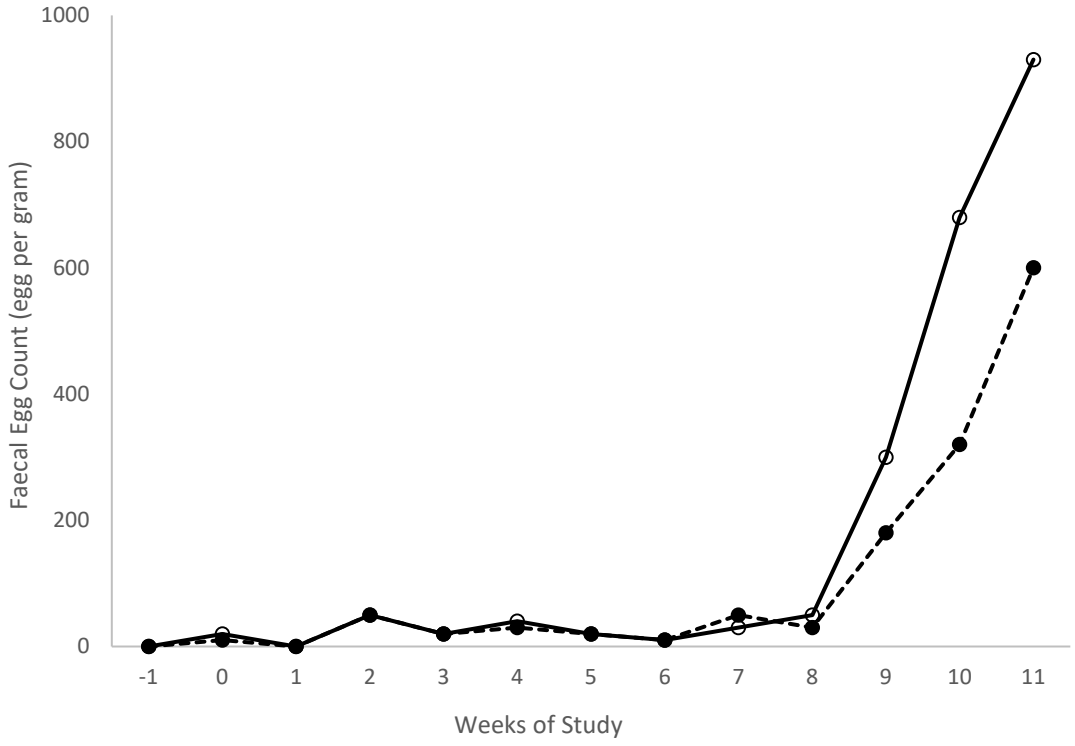


**Figure 2.2: Faecal egg count (FEC) for lambs supplemented with dietary vitamin E either at 5.3 IU/kg BW/day (○) or 10 IU/kg BW/day (●) for a period of 11 weeks**

Source: De wolf *et al* (2014)

Therefore, it could be interesting to elucidate the rate at which the performance of lambs supplemented with dietary vitamin E may be influenced when supplied an adequate amount of dietary protein supplementation. Similarly, there is no information on the interaction of dietary protein and vitamin E supplementation on GIN naturally infected lambs. Elucidating the interaction of dietary protein and vitamin E supplementation on growth, FEC and haematological performance is of essence since the effect of parasitism is detrimental to lamb health and production. Supplementing both dietary protein and vitamin E may improve lamb performance, rather than one or none of both.

## 2.5 Summary

The deteriorating influence of GIN to sheep health and productivity cannot be overemphasized. Monitoring GIN measures in lamb explains the impact of nematodiosis on growth performance and blood profile. Pasture management may reduce the rate of reinfection and pasture contamination. Climate change and other associated factors may render such preventive method void. Anthelmintic treatment was introduced to combat parasitism. However, intestinal nematodes are resistant to all classes of anthelmintics. The body mechanisms that enhance growth and immune performance are proteinaceous in nature and antioxidant function against free radical production in tissue, organ and cell damages. Thus, the influence of dietary protein and vitamin E supplementation in improving lamb productivity and health status cannot be underestimated. Therefore, the need to explore the interaction of dietary protein and vitamin E supplementation in GIN naturally infected lambs. The broad objective of the current study is to assess the interaction of dietary protein and vitamin E supplementation on faecal egg counts, growth performance and haematological indices in gastrointestinal nematode naturally infected lambs.

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# CHAPTER 3

## Interaction of dietary protein and vitamin E supplementation on faecal egg counts and growth performance in lambs


### Abstract

The interaction of dietary protein and vitamin E supplementation was assessed on faecal egg counts (FEC) and growth performance of lambs. The lambs were allocated to each of the four treatment combinations, with six lambs in each treatment group. Treatment one lambs received dietary protein and vitamin E (PE), Treatment two lambs received dietary protein and no vitamin E (PNE), and Treatment three received dietary vitamin E and no protein (NPE), and Treatment four received no dietary protein and vitamin E supplementation (NPNE). The trial ran for eight weeks. The lambs were allowed to graze on *Pennisetum clandestinum* contaminated with a heavy load of nematodes. Dietary protein supplementation increased ($P<0.01$) average daily gain (ADG) and body condition scores (BCS). Dietary vitamin E supplementation had no effect on ADG and BCS. There was no interaction ($P>0.05$) between dietary protein and vitamin E supplementation on ADG and BCS. There was an interaction ($P<0.01$) between dietary protein and vitamin E supplementation on faecal egg counts (FEC), cultured *Haemonchus* larvae ($CHL_3$) and cultured *Trichostrongylus* larvae ($CTL_3$). Lambs in the PE and PNE treatment had lower ($P<0.05$) FEC, $CTL_3$ and $CHL_3$, than NPE and NPNE l treatment lambs, which had similar but higher ($P>0.05$) FEC, $CTL_3$ and $CHL_3$. It could be concluded that there was an interaction between dietary protein and vitamin E supplementation on FEC, $CHL_3$ and $CTL_3$ in lambs.



**Keywords**: gastrointestinal nematodes, average daily gain, body condition scoring, *Haemonchus*, *Trichostrongylus*, larvae culturing

## 3.1 Introduction

Gastrointestinal nematodes (GIN) hinder the performance of lambs, resulting in serious economic losses (Abebe *et al*., 2010). Increased faecal egg counts (FEC) and body weight losses are associated pathophysiological consequences of a parasited host. Gastrointestinal nematodes make the infested animal unable to efficiently utilize dietary nutrients, especially, when feed quality and quantity is low (Xhomfulana *et al*., 2009). Lamb vulnerability to nematode infestation and infection also increases. *Haemonchus* and *Trichostrongylus* are the common nematode species that affect sheep (Van Wyk *et al*., 2004). Farmers usually use anthelmintics to control nematodes.

In lambs, nutrients are needed for growth, reproduction and immunity development. Importantly, dietary protein and vitamin E regulates intestinal tissue and cell functioning (Khan *et al*., 2012; Anugu *et al*., 2013). Mature GIN in the gut compromises the absorption of the little nutrients available. Dietary supplementation with essential nutrients in such cases may be necessary to avert GIN infestation. Dietary protein supplementation increase sheep performance and reduce GIN fecundity in lambs (Louvandini *et al*., 2006). Dietary vitamin E supplementation reduce FEC in lambs (De wolf *et al*., 2014). The use of dietary vitamin E supplementation to boost susceptible lamb performance may depend on the status of dietary protein in host.

The overuse of anthelmintics resulted into nematode resistance (Fleming, 2006). Therefore, the need for an alternative method of approach to control parasitism. Anthelmintic resistance, loss of body weights and condition and increased FEC in grazing lambs are constraint. Also, poor nutrient quality of *Pennisetum clandestinum* presents the need to explore dietary protein and vitamin E supplementation in susceptible lambs on FEC and growth performance. Interaction

of dietary protein and vitamin E supplementation may present a promising nutritional approach of controlling parasitism, through reduced GIN pathogenesis and fecundity, and minimizes rate of reinfection, hence, abate pasture contamination. Lamb resistance to anthelmintics, increase in GIN egg excretion, reinfection, and pasture contamination necessitates exploring the interaction of dietary protein and vitamin E supplementation on FEC and growth performance in lambs.

The objective of the study was to assess the interaction of dietary protein and vitamin E supplementation on FEC and growth performance in lambs. It was hypothesized that, there is an interaction between dietary protein and vitamin E supplementation on FEC and growth performance in lambs.

## 3.2 Materials and methods

### 3.2.1 Ethical considerations of the study

The experimental procedures were done according to the ethics rules and regulations, specified by the Certificate of Authorization to Experiment on Living Animals given by Animal Ethics Committee, UKZN (Reference number: AREC/096/015M).

### 3.2.2 Description of the study site

The study was conducted at the Livestock section of Ukulinga Research Farm, University of KwaZulu-Natal, Pietermaritzburg, South Africa. It lies on the geographical coordinate's 29° 39' 49.9284" S and 30° 24' 14.6628" E at an altitude of about 700 m. Maximum temperature in the summer is around 29°C with minimum temperature averaging around 16°C. In extreme

cases, summer temperature may rise above 33°C with minimum temperatures as low as 7°C at night in winter. The rainfall pattern is characterized by an annual rainfall of approximately 730 mm, which normally falls mostly in the summer between October and April.

### 3.2.3 Experimental lambs

Twenty four (12 male and 12 female) Dohne Merino lambs aged about 12 months, with mean body weight of 20.21 kg were used for the experiment. The lambs were ear-tagged for identification. All the lambs were drenched at the beginning of the trial with First Drench® anthelmintic (containing a combination of Abamectin and Praziquantel) following the recommended dosage for sheep at 2.5 mL/10 kg body weight (BW). The anthelmintic was administered orally using a dosing gun.

### 3.2.4 Treatments

Lambs were allocated to each of the four treatment combinations, with six lambs of both sexes in each treatment group. The experimental lambs were acclimatized for 10 days to adapt to their respective diets. The experiment was done using a 2 × 2 factorial arrangement, with two factors. Treatment 1 lambs were supplemented with dietary protein and vitamin E supplementation (PE). Treatment 2 lambs were supplemented with dietary protein but no vitamin E supplementation (PNE). Lambs in both PE and PNE treatment groups were given 150 g of soyabean meal, daily. Treatment 3 lambs were supplemented with dietary vitamin E and no protein supplementation (NPE). Treatments PE and NPE lambs were drenched a powdered form of vitamin E (dl-α-tocopherol) dissolved in 100 ml clean fresh water at 30 IU/kg BW/every seven days using a dosing gun throughout the experimental period. Treatment 4 lambs were maintained on *Pennisetum clandestinum* pasture. Lambs in NPNE neither received dietary protein nor vitamin E supplement.

### 3.2.5 Housing and management

All the lambs were released to graze freely on contaminated *Pennisetum clandestinum* between 0900 – 1500 h, daily to continue their exposure to infective nematode larvae ($L_3$). The experiment was conducted between March – May. The grass harboured between 315 – 677 nematode $L_3$ larvae/kg of dry herbage, with a mean of 425 ± 174 nematode $L_3$ larvae/kg of dry herbage (Mawahid, 2013). Clean fresh water was given to the lambs *ad libitum* on the veld and the sheep pen house. All sheep were housed at night and kept in an individual sheep pen (1.5 × 1 m). The sheep house was raised and well ventilated and lights were switched on at night for easy access to feed troughs. A digital scale (METTLER Ch-8606 – Zurich, Switzerland) was used to measure the quantity of protein and vitamin E supplied to treatment lambs, daily. Plastic trays were placed under each feed troughs to collect feed spillages. The feed spilled were dried weighed and discarded daily.

### 3.2.6 Chemical analyses of *Pennisetum clandestinum*

The herbage plot (*Pennisetum clandestinum*) was sub-divided into six plots (each plot area of 4046 $m^2$). Lambs were grazed rotationally on the sub-divided plots. Grass was green throughout the experiment. Herbage sample was collected from each plot at the beginning of the trial for chemical analyses. The collected herbage samples were mixed together, washed to remove debris, dried, and then pulverized with soyabean meal sample (Glen Creston Stanmore, England) through a 1.0 mm diameter sieve. The samples were then analysed for chemical composition.

Dry matter (DM), crude protein (CP) and ash were determined following the method of AOAC (1995). The Dumas combustion method (LECO Truspec nitrogen analyser, St Joseph MI, USA) was used to determine the nitrogen (N) content of feed samples and the CP content was

calculated by multiplying the total N by a factor of 6.25. Soyabean meal and herbage samples were weighed into a dry porcelain dish and heated in a muffle furnace at 600° C for 12 hours, after which desiccated before weighing. The ash percentage was then calculated. Neutral detergent fiber (NDF) and acid detergent fibre (ADF) were analysed using ANKOM Fibre Analyser (Ankom Macedon, NY, USA) according to Van Soest *et al*., (1991). The NDF was assayed using heat stable α-amylase (Sigma A3306; Sigma Chemical Co., St Louis, MO, USA) (Table 3.1).

### 3.2.7 Measurements

#### 3.2.7.1 Body weights

Body weights were recorded using (RUUDWEIGH, KM-2E, Ruudscale Durban, South Africa) electronic weighing scale and each lamb was weighed and recorded once every week at 0800 h before the lambs were supplied with feed.

**Table 3. 1: Chemical properties of *Pennisetum clandestinum* and Soyabean meal**

| | | (g/kg DM) |
|---|---|---|
| Item | *Pennisetum clandestinum* | Soyabean meal |
| Dry matter | 94 | 86.6 |
| Ash | 2.54 | 2.71 |
| Crude protein | 10.13 | 47.17 |
| Acid detergent fibre | 5.18 | 27.89 |
| Neutral detergent fibre | 6.16 | 13.06 |

### 3.2.7.2 Body condition scoring

Body condition scoring (BCS) (Gerhart *et al*., 1996) was assessed to determine the nutritional status of each lamb. Body condition scoring was done by determining the amount of fat covering, over and around the lumbar vertebrae, rib cage and sternum area of each lamb. Moreso, the spinous process around each lamb back behind the last rib in front of the hip bone were felt carefully with the hands. The spinous process was assessed to determine the condition of the ridge around the spine and the level of sharpness. The process was repeated once every week (Table 3.2).

### 3.2.8 Nematode faecal egg counts

Faeces were collected on day 0, 7, 14, 21, 28, 35, 42, 49 and 56. Samples were collected directly from the rectum of lambs, placed in transparent plastic bags and tagged by lamb's identification, then transferred to the Animal and Poultry Science Laboratory, University of KwaZulu Natal, Pietermaritzburg where faecal analyses for nematode egg parasite counts were conducted. Faecal samples were collected between 0830 and 0900 h.

**Table 3. 2 Body condition scoring scale used in the study**

| Condition scale | Body condition score |
|---|---|
| 1 | Sharpness at the transverse processes, the fingers pass easily under the ends, process are easily felt.<br>Lamb ribs are visible. |
| 2 | Spinous processes feel prominent but smooth and transverse processes are smooth and rounded, and the fingers pass under the ends with slight pressure.<br>There is little amount of fat cover and ribs are still easily felt. |
| 3 | The transverse processes are smooth and well covered, and firm pressure is required to feel over the ends.<br>Ribs are barely visible; an even layer of fat covering them. Spaces between ribs are felt with pressure. |
| 4 | The ends of the transverse processes cannot be felt. The eye muscle areas are full, and have a thick covering of fat.<br>Ribs not visible. |
| 5 | The spinous process cannot be detected even with pressure. Transverse processes are undetectable. There may be large deposits of fat over the rump and tail.<br>Ribs not visible due to excess fat. |

(Gerhart *et al*., 1996)

Nematode egg count was done for each sample using the McMaster Technique, as described by Hansen and Perry (1994). 58 ml of saturated sugar solution was added to 2 g of each crumbled faecal sample in a beaker. The solution was then mixed thoroughly with a BRAUN MQ100 hand blender and was filtered through a 1.5 mm diameter sieve into a second container. Few drops of amyl alcohol was then added to the filtrate to reduce the bubbles, after then stirred with a Pasteur pipette and a sub sample was withdrawn with a Pasteur pipette.

Each chamber of the McMaster double chamber slide was filled with the fluid. The chambers were allowed to stand for two minutes and the fluid were examined using a light microscope at 10 × magnification. The eggs within each grid of the McMaster double chamber counting slides were then multiplied × 100 to give the egg per gram (epg) in faeces. The eggs were counted and identified. Nematode eggs are relatively small and can easily be viewed under a light microscope, *Haemonchus* eggs are yellowish in colour, with an ovular shape and much bigger compared to other nematode egg specie. *Trichostrongylus* are circularly shaped and relatively smaller with a light brownish colour (Van Wyk *et al*., 2004).

### 3.2.9 Nematode egg culturing and harvesting

Faecal samples from the treatment lambs was mixed and pooled into four beakers, each for the treatment groups, then 5 g sub faecal samples was processed into four petri dishes each for the treatment groups to make a combination of sixteen samples. These samples were then placed in an incubator (MEMMERT, 854 Schwabach, West Germany) at 27° C for 12 days. Vermiculites were sprinkled around the samples and damped every day at 0700h to keep it moist throughout the incubation period.

The Baermann culture technique, as described by Hansen and Perry (1994), was used to harvest, count and identify nematode $L_3$ larvae species. On day 13, each faecal culture was placed in a double layer cheesecloth and tied using a rubber band. The tied cultured samples were then placed in a funnel, supported by a funnel stand. The material was filled with lukewarm water until it covered the faecal culture. The samples were left to stand for 24 hours. Then, 15 ml of the fluid was collected from the bottom of the tied funnel into a 20 ml test tube and the tubes were allowed to stand for 30 minutes. Aliquots were withdrawn with a Pasteur pipette and few drops were transferred onto a microscope slide. A drop of iodine was added to the substance to enhance clear visibility of the $L_3$ stage of nematode species, then covered with a cover slip. The samples were examined, identified and $L_3$ larvae were counted at 10 × magnification using a light microscope. The predominant specie of nematode present was identified according to Van Wyk *et al*. (2004). Faecal culturing was only done at the end of the experiment.

### 3.2.10 Statistical analyses

The PROC MIXED procedure of SAS (2010) was used to analyse ADG, BCS and FEC data. The PROC GLM procedure of SAS (2010) was used to analyse data for cultured *Haemonchus* $L_3$ ($CHL_3$) and cultured *Trichostrongylus* $L_3$ ($CTL_3$). Faecal egg count data were checked for normality using PROC UNIVARIATE (SAS, 2010). The data were then logarithmic transformed $Log_{10}(\chi + 1)$ to confer normality in the distribution. Comparison of least square means was done using the PDIFF option of SAS (2010). The model used was:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \lambda_k + \delta_l + (\alpha \times \beta)_{ij} + (\alpha \times \lambda)_{ik} + (\beta \times \lambda)_{jk} + (\alpha \times \beta \times \lambda)_{ijk} + \varepsilon_{ijkl};$$

Where; $Y_{ijkl}$ = response variable (ADG, BCS, FEC);

$\mu$ = population mean;

$\alpha_i$ = dietary protein supplementation;

$\beta_j$ = dietary vitamin E supplementation;

$\lambda_k$ = week;

$\delta_l$ = sex;

$(\alpha \times \beta)_{ij}$ = interaction between dietary protein and vitamin E supplementation;

$(\alpha \times \lambda)_{ik}$ = interaction between dietary protein and week;

$(\beta \times \lambda)_{jk}$ = interaction between dietary vitamin E and week;

$(\alpha \times \beta \times \lambda)_{ijk}$ = interaction between protein, vitamin E and week;

$\varepsilon_{ijkl}$ = standard error.

Faecal culturing model used in the experiment:

$Y_{ijk} = \mu + \alpha_i + \beta_j + \lambda_k (\alpha \times \beta)_{ij} + \varepsilon_{ijk}$;

$Y_{ijk}$ = response variable ($CHL_3$ and $CTL_3$)

$\mu$ = population mean;

$\alpha_i$ = dietary protein supplementation;

$\beta_j$ = dietary vitamin E supplementation;

$\lambda_k$ = sex;

$(\alpha \times \beta)_{ij}$ = interaction of dietary protein and vitamin E supplementation;

$\varepsilon_{ijk}$ = standard error.

## 3.3 Results

### 3.3.1 Average daily gain

Lambs that received dietary protein supplementation had higher ADG. Dietary vitamin E supplementation did not affect ADG. There was a significant effect of week on ADG. Sex of lambs did not affect ADG. There was no interaction of dietary protein and week on ADG. There was no interaction between dietary protein, vitamin E and week on ADG (Table 3.3). There was no interaction ($P>0.05$) between dietary protein and vitamin E supplementation on ADG in lambs. As shown in Table 3.4, lambs in the PE and PNE treatment had similar and higher ADG than NPE and NPNE treatments, which had similar but lower ADG.

### 3.3.2 Body condition scoring

Dietary protein supplementation affected BCS in lambs. Lambs that received dietary vitamin E supplementation had lower BCS than those without. There was a week effect ($P<0.01$) on BCS in lambs. Sex of lambs did not affect BCS. The interaction of dietary protein and week had no effect on BCS. There was no interaction between dietary vitamin E and week on BCS. There was no interaction of dietary protein, vitamin E and week on BCS in lambs (Table 3.3). There was no interaction ($P>0.05$) between dietary protein and vitamin E supplementation on BCS. As shown in Table 3.4, lambs in the PE and PNE treatment had similar and higher BCS whereas, NPE and NPNE treatment lambs had similar but lower BCS.

### 3.3.3 Faecal egg counts

Dietary protein and vitamin E supplementation did not affect FEC in lambs. Sex and week had no effect on FEC in lambs. There was no interaction between dietary vitamin E and week on FEC. There was no interaction between dietary protein and week on FEC. There was no

interaction of dietary protein, vitamin E and week on FEC in lambs. There was an interaction ($P<0.01$) between dietary protein and vitamin E supplementation on FEC in lambs (Table 3.3). Lambs in the PE and PNE treatment had lower ($P<0.05$) FEC compared to NPE and NPNE lambs, which had similar ($P>0.05$) FEC (Table 3.5). The PNE, NPE and NPNE treatment lambs had an increased FEC but PE treatment lambs consistently had a lower FEC as the week progressed (Figure 3.1).

### 3.3.4 Cultured *Haemonchus* $L_3$ larvae and cultured *Trichostrongylus* $L_3$ larvae

Dietary protein supplementation did not affect $CHL_3$ in lambs. Lambs that received dietary vitamin E supplementation had lower $CHL_3$ than those without. There was no sex effect on $CHL_3$ in lambs. There was an interaction ($P<0.01$) between dietary protein and vitamin E supplementation on $CHL_3$ (Table 3.3). Lambs in the PNE and NPNE treatment had higher ($P>0.05$) $CHL_3$ whereas, PE and NPE treatment lambs had fewer ($P<0.05$) $CHL_3$ (Table 3.5). Dietary protein and vitamin E supplementation affected $CTL_3$ in lambs. Sex of lambs had no effect on $CTL_3$. The effect of week and the interaction of dietary protein, vitamin E and week was not investigated on $CHL_3$ and $CTL_3$. There was an interaction ($P<0.05$) between dietary protein and vitamin E supplementation on $CTL_3$ (Table 3.3). The PE and PNE treatment lambs had lower ($P<0.05$) $CTL_3$ whereas, NPE and NPNE treatment had similar ($P>0.05$) $CTL_3$ (Table 3.5).

**Table 3. 3: Level of significance for average daily gain (ADG), body condition score (BCS), faecal egg count (FEC), cultured *Haemonchus* larvae ($CHL_3$), and cultured *Trichostrongylus* larvae ($CTL_3$) in lambs**

| Effects | Traits | | | | |
|---|---|---|---|---|---|
| | **ADG** | **BCS** | **FEC** | **$CHL_3$** | **$CTL_3$** |
| Protein | * | ** | NS | NS | * |
| Vitamin E | NS | NS | NS | * | * |
| Week | ** | ** | NS | - | - |
| Sex | NS | NS | NS | NS | NS |
| Protein × vitamin E | NS | NS | * | * | * |
| Protein × week | NS | NS | NS | - | - |
| Vitamin E × week | NS | NS | NS | - | - |
| Protein × vitamin E × week | NS | NS | NS | - | - |

*P<0.05;**P<0.001; NS: Not significant (P>0.05); - : not investigated because nematode specie was only identified at the end of the trial.

**Table 3. 4: Least square means (± standard error) for interaction between dietary protein and vitamin E supplementation on average daily gain and body condition scoring in lambs**

| Supplements | | ADG (g/day) | |
|---|---|---|---|
| | | **E** | **NE** |
| | **P** | 68.8 ± 15.4[b] | 58.8 ± 13.8[b] |
| | **NP** | 18.7 ± 14.2[a] | 16.8 ± 13.8[a] |
| | | **BCS** | |
| | | **E** | **NE** |
| | **P** | 4.6 ± 0.10[b] | 4.5 ± 0.09[b] |
| | **NP** | 3.6 ± 0.10[a] | 3.7 ± 0.12[a] |

[ab] Values with different superscripts differ significantly (P<0.05).

P: protein; NP: no protein; E: vitamin E; NE: no vitamin E.

ADG: average daily gain; BCS: body condition scores; g/day: grams per day

**Table 3. 5: Least square means (± standard error) for interaction between dietary protein and vitamin E supplementation on faecal egg count, cultured *Haemonchus* larvae and cultured *Trichostrongylus* larvae in lambs**

| | | FEC (epg) | |
|---|---|---|---|
| **Supplements** | | **E** | **NE** |
| | **P** | $2.32 \pm 0.19^{a}$ | $3.07 \pm 0.14^{b}$ |
| | **NP** | $3.09 \pm 0.16^{b}$ | $2.87 \pm 0.16^{b}$ |
| | | **$CHL_3$** | |
| | | **E** | **NE** |
| | **P** | $3.25 \pm 1.23^{a}$ | $11.0 \pm 1.23^{b}$ |
| | **NP** | $10.0 \pm 1.23^{b}$ | $8.5 \pm 1.23^{b}$ |
| | | **$CTL_3$** | |
| | | **E** | **NE** |
| | **P** | $4.75 \pm 2.05^{a}$ | $13.75 \pm 2.05^{b}$ |
| | **NP** | $14.75 \pm 2.05^{b}$ | $14.75 \pm 2.05^{b}$ |

[ab] Values with different superscripts differ significantly ($P<0.05$).

P: protein; NP: no protein; E: vitamin E; NE: no vitamin E.

FEC: faecal egg counts; epg: egg per gram of faeces; $CHL_3$: cultured *Haemonchus* $L_3$ larvae; $CTL_3$: cultured *Trichostrongylus* $L_3$ larvae

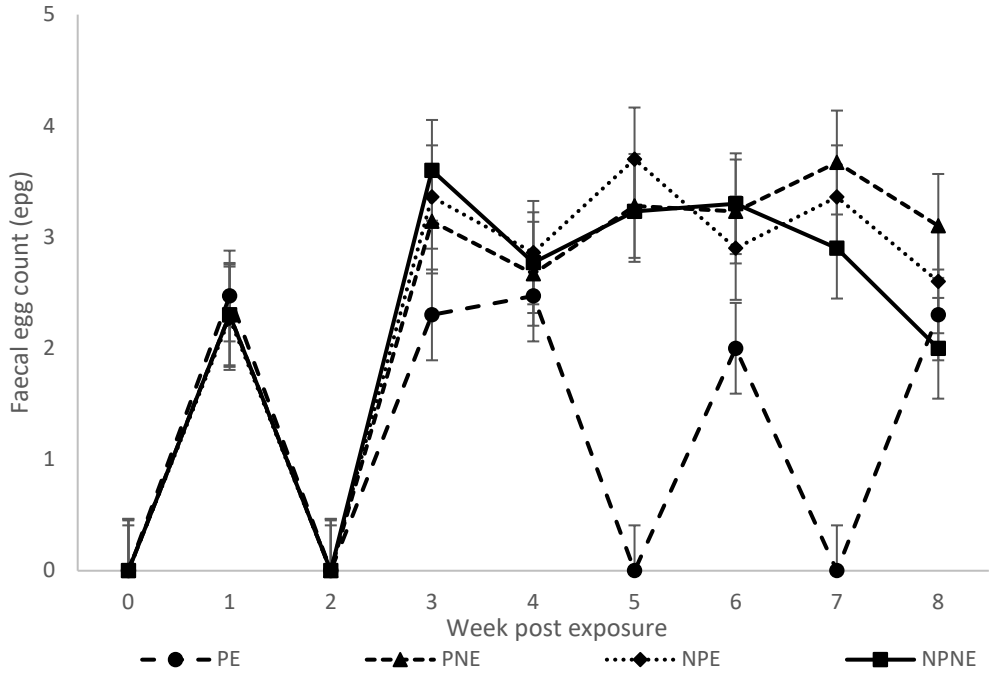


**Figure 3.1: Effect of dietary protein and vitamin E supplementation on week post exposure (LSM ± SE) on FEC in lambs**

PE: protein and vitamin E; PNE: protein and no vitamin E; NPE: vitamin E and no protein; NPNE: no protein and no vitamin E; LSM: least square mean; SE: standard error.

### 3.4 Discussion

The high risk and poor pasture management of susceptible lambs grazed on heavily contaminated *Pennisetum clandestinum* coupled with anthelmintics resistance triggered the emergence need for an alternative method of controlling parasitism. Tembely *et al*. (1999) and Mawahib (2013), highlighted that the nematode $L_3$ larvae/kg of dry herbage from *Pennisetum clandestinum* were high enough to contaminate the pasture, especially, under a stabilized weather condition which may favour the survival of nematodiosis. These increase lamb vulnerability and sustainability to parasites. The possibilities of combating gastrointestinal nematodes have been explored, through dietary protein supplementation (Khan *et al*., 2012; Mhomga *et al*., 2012). The use of dietary vitamin E supplementation has been proposed, however, its effectiveness in improving lamb performance may depend on dietary protein supplementation.

The hypothesis tested was that there was an interaction between dietary protein and vitamin E supplementation on FEC and growth performance in lambs. In the present study, the increased ADG in dietary protein supplemented lambs were similar to Xhomfulana *et al*. (2009) and Khan *et al*. (2012), who reported an improved ADG in GIN mixed infection. The increase in ADG for lambs supplemented dietary protein could be as a result of high crude protein content in the diet which provide lambs with adequate amino acids needed for growth and maintenance. The present study highlighted the effect of dietary vitamin E supplementation on ADG in lambs, and dietary vitamin E supplementation had no influence on ADG. The performance of lamb supplied with dietary vitamin E supplementation may be due to inadequate nutrient composition of the pasture. Supplementing both dietary protein and vitamin E in the present study may not be significantly affect ADG. However, ADG value for PE treatment lambs was higher compared to the PNE, NPE and NPNE treatment lambs.

Similarly, assessing the BCS of a parasite infected host plays a significant tool in lamb health, since it measures body reserves (Gerhart *et al*., 1996). The significant result observed on BCS in the present study for lambs supplied with dietary protein agrees with Xhomfulana *et al*. (2009) and Louvandini *et al*. (2006), who reported an increase in BCS when supplementing a diet high in protein under natural infection. The performance displayed by lambs that received dietary protein confirmed that the nutrient was adequate enough for maintenance of BCS in host (Louvandini *et al*., 2006). The non-significant result on dietary vitamin E supplementation on BCS in the present findings proved that supplementing infected lambs with dietary vitamin E may not be sufficient to improve the BCS of infected lambs.

Several reports recorded a significant response when supplementing GIN infected lambs with dietary protein (Louvandini *et al*., 2006; Baker *et al*., 2002). Few, if any, had reported using a direct source of protein for that purpose in elucidating the extent at which dietary protein supplementation can influence a reduction in FEC. In the present study, soyabean meal - a high protein source was used. The effect of dietary protein on FEC in the study agrees with the findings of Khan *et al*. (2012), who used compounded feed and recorded no significant effect on FEC when lambs were supplied a diet high in protein. The reason for the result on dietary protein supplementation in the present study may be due to the mixed challenged infection with *Haemonchus* and *Trichostrongyl*us, which then makes it difficult for lambs to effectively and adequately utilize nutrients, since *Haemonchus* and *Trichostrongyl*us activities causes hypoproteinaemia and diarrhoea (Van Wyk *et al*., 2004).

Similarly, the finding obtained on dietary vitamin E supplementation on FEC corresponds with MacGlafin *et al*. (2011) and De wolf *et al*. (2014), who reported no significant effect of dietary vitamin E supplementation on FEC in lambs. Nevertheless, the effectiveness of dietary protein

supplementation in reducing FEC may depend on dietary vitamin E supplementation, as seen in Table 3.3 and Table 3.5. In addition, lambs that received dietary protein and vitamin E supplementation tend to have less FEC than those without. Response of infected lambs in the study may be as a result of dietary protein supplementation, which stimulates the gut against matured GIN (Simeone *et al*., 2005) and dietary vitamin E supplementation, which then abate GIN injuries from the intestine (Larsen *et al*., 1988). However, the extent at which dietary protein supplementation interacts with dietary vitamin E supplementation is unknown.

The influence of dietary protein and vitamin E supplementation was observed on week post exposure. Result obtained from the present findings on FEC shows that, dietary vitamin E and protein supplementation were unable to function independently, as observed on FEC for dietary protein and vitamin E supplementation which increased throughout the week post exposure. There was an increasing on FEC for PNE and NPE lambs as the week of exposure progressed which may be due to the mixed infection of GIN, contrary to Louvandini *et al*. (2003), who reported that dietary protein enhanced the sheep ability to withstand a mixed GIN infection. The PE lambs proved a dependent influence on both dietary protein and vitamin E supplementation. The PE treatment lambs consistently excreted fewer GIN eggs as the week progressed.

Similarly, nematode $L_3$ culturing was done to identify the predominant specie of GIN affecting lambs, since the parasites were acquired under natural grazing condition. The effect of dietary protein on $CHL_3$ agrees with Khan *et al*. (2012) and the response of lambs in the present study may be associated to the age of lambs and the natural parasite acquiring method adopted in the present study. However, the influence of dietary protein supplementation on $CTL_3$ is not understood, since both GIN specie resides in the intestinal compartment of host and the diet

was expected to have the same effect on GIN specie, hence, there is a need to explore the mechanism of dietary protein activities on $CTL_3$.

In the present study, dietary vitamin E had an influence on $CHL_3$ and $CTL_3$ but contrary to De wolf *et al*. (2014), who investigated the effect of dietary vitamin E supplementation on experimental *Haemonchus* infection. Lambs in the present study were given dietary vitamin E orally at 30 IU/kg BW/day as compared to the study by MacGlafin *et al*. (2011). The poor performance of lambs in the study by MacGlafin *et al*. (2011) may be associated with the insufficient amount (15 IU/kg BW/day) of dietary vitamin E supplied to the treatment lambs and the method of administration adopted in the study. Treatment lambs in the present study were drenched orally, since dietary vitamin E is absorbed in the gut (Rehim *et al*., 2003). However, for an effective performance of dietary protein on $CHL_3$ and $CTL_3$, treatment lambs may need 30 IU/kg BW/day of dietary vitamin E to minimize $CHL_3$ and $CTL_3$ count (Table 3.3 and 3.5).

The interaction of dietary protein and vitamin E supplementation was observed on $CHL_3$ and $CTL_3$ in Table 3.3 and Table 3.5. Results from the present study on lamb response on $CHL_3$ and $CTL_3$ may be due to adequate amount of dietary protein and vitamin E received by treatment lambs. Again, the effectiveness of dietary protein and vitamin E supplementation may depend on each other. Therefore, the response of treatment lambs to combat $CHL_3$ and $CTL_3$ may be due to their ability to effectively convert and utilize consumed nutrient in the gut. Culturing of *Haemonchus* and *Trichostrongylus* $L_3$ were only investigated once since they are the predominant specie of nematode found in the area (Tembely *et al*., 1999; Mawahib, 2013).

Treatment lambs in the present study were exposed to an uncontrolled amount of parasitic infection, unlike, MacGlafin *et al*. (2011) and Khan *et al*. (2012) who assessed the influence of dietary protein and vitamin E supplementation on experimentally infection in lambs. Elucidating the interaction of dietary protein and vitamin E supplementation on GIN naturally infected lambs is important, since most farming practises are practically extensive. Therefore, lambs maintained under an extensive grazing practises may require high nutritional demand to challenge parasitism, especially in the tropics, where forage quality and availability diminishes, and such methods observed under full confinement and a specific level of infection are indispensable.

### 3.5 Conclusions

The present study confirmed the interaction between dietary protein and vitamin E supplementation on FEC, *Haemonchus* and *Trichostrongylus* larvae in lambs. Therefore, the effectiveness of dietary vitamin E supplementation on faecal egg counts, *Haemonchus* larvae and *Trichostrongylus* larvae is determined by adequate amount of dietary protein supplementation in lambs. Nonetheless, these warrant elucidating the interaction of dietary protein and vitamin E supplementation on cells and immune performance against GIN infected lambs.

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# CHAPTER 4

## Interaction of dietary protein and vitamin E supplementation on haematological indices in lambs


### Abstract

The interaction of dietary protein and vitamin E supplementation on haematological indices and FAMACHA scores in lambs was assessed. Lambs aged about 12 months were randomly assigned into four treatment groups of six lambs each. Treatment 1 lambs received dietary protein and vitamin E (PE), treatment 2 lambs received dietary protein and no vitamin E (PNE), and treatment 3 received dietary vitamin E and no protein (NPE), and treatment 4 received neither dietary protein nor vitamin E supplementation (NPNE). The lambs grazed on *Pennisetum clandestinum* contaminated with a heavy load of nematodes. FAMACHA scores were used to assess the health status of lambs. Blood samples were collected for haematological indices. Dietary protein supplementation increased ($P<0.05$) packed cell volume (PCV), haemoglobin (Hb), erythrocyte (RBC) and mean corpuscular haemoglobin concentrations (MCHC), and reduced ($P<0.05$) eosinophil concentrations and FAMACHA scores in lambs. Dietary vitamin E supplementation increased ($P<0.05$) mean corpuscular haemoglobin (MCH), MCHC, lymphocytes and decreased eosinophil concentration ($P<0.05$). There was an interaction ($P<0.05$) between dietary protein and vitamin E supplementation on eosinophil concentrations and FAMACHA scores. It could be concluded that dietary protein and vitamin E supplementation improves lamb health status.



**Keywords:** gastrointestinal nematodes, eosinophils, lymphocytes, FAMACHA

## 4.1 Introduction

Gastrointestinal nematodes (GIN) are one major factor limiting livestock production (Van Wyk *et al*., 1997). Despite the much focus on its epidemiology, they still pose a great threat to lamb health (Vlassoff *et al*., 2001). The adult nematode mounts the intestinal wall of host and they cause major discomfort to the host. A widespread of nematode species are found in southern Africa. *Haemonchus* are blood sucking parasites. Parasites cause anaemia, abnormal increase or decrease in lymphocyte and eosinophil concentration, and diarrhoea (Sharma *et al*. 2000).

Over a decade, small resource farmers adopted the use of FAMACHA to cheaply and physically identify anaemia in ruminant, especially sheep (Van Wyk, 2002; Kaplan, 2004). Ruminants maintained on a poor diet deficient in dietary protein and vitamin E supplementation are vulnerable to GIN. Parasitic infection may hinder sheep ability to efficiently utilize nutrients (Simone *et al*., 2005). Lambs fed on a ration containing high proteins had reduced perilous parasitic infection due to a high immune response (Strain, 2001). Dietary vitamin E supplementation enhances a responsive immune system of sheep (Anugu *et al*., 2013). The effective response of susceptible lamb fed dietary vitamin E supplementation may depend on dietary protein supplementation to improve lamb health and combat parasitism. As seen in Chapter 3, the interaction of dietary protein and vitamin E supplementation subsided GIN pathophysiological consequences.

Anthelmintic control has consistently been used to combat nematodiosis (Wolstenholme *et al*., 2004). The continuous use of various classes of anthelmintics has led to nematode resistance to synthetic therapy (Fleming *et al*., 2006). Little, if any had recently been reported about the interaction of dietary protein and vitamin E supplementation on haematological indices in lambs. Anaemia and low erythrocyte concentration are associated constraints. Supplementing

dietary protein and vitamin E may improve lamb health status, especially when grazed on GIN contaminated pasture and low forage quality. Monitoring FAMACHA scores, packed cell volume (PCV), haemoglobin (Hb), erythrocytes (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin count (MCHC) leucocytes (WBC), lymphocyte concentration, and eosinophil concentration may help elucidate GIN impact on blood profile.

The objective of the study was to assess the interaction of dietary protein and vitamin E supplementation on haematological indices and FAMACHA scores in lambs. It was hypothesized that there was an interaction between dietary protein and vitamin E supplementation on haematological indices in lambs.

## 4.2 Materials and methods

### 4.2.1 Ethical considerations and study site

A detailed description of the study site, average temperature and relative humidity and ethics approval for use and management of experimental lambs are described in section 3.2.1.

### 4.2.2 Experimental design, treatment diets and sheep management

Description of experimental lambs, dietary treatments, diet composition, lamb housing and management are given between sections 3.2.2 and 3.2.6.

### 4.2.3 Data collection

#### 4.2.3.1 FAMACHA

The FAMACHA technique was used to assess the health status of treatment lambs. The technique was used following the procedure outlined by Van Wyk (2002). Each lamb was restrained and held between the examiners legs, then the upper thumb of the right hand was placed gently on the right eye of each lamb, then the left thumb was used to pull down the lower eyelid of lamb and the colour of the mucous membrane of the lower eyelid was assessed and scored using FAMACHA (Table 4.1) (Van Wyk, 2002). The lambs were examined between 0930 and 1000 h. The trial ran for eight weeks.

#### 4.2.3.2 Haematological indices

Ethylene diamine tetra-acetic acid (EDTA) tubes were used to collect 5 ml non-clotted whole blood through jugular venepuncture for haematological examination. Haematological measurements were done at Vetdiagnostix centre, Cascades, Pietemaritzburg, KwaZulu Natal, South Africa. Blood samples were analysed within 24 h of blood collection. Lambs were bled at 0900 h on day 56 of the experiment. The Symex, 2000 xn analyser was used for haematology. Packed cell volume (PCV) was measured using a haemocrit reader through a micro-haematocrit capillary tube. Erythrocyte count (RBC), and haemoglobin (Hb) counts was measured using the Hydro-Dynamic Focus Detector. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) was also measured. The flow cytometry with three angle laser scatter analyser was used to count and differentiate the fractions of leucocytes (WBC), lymphocytes and eosinophils.

**Table 4. 1: FAMACHA scoring table used in the study**

| Rating | Classification (Colour) | Comment |
|---|---|---|
| 1 | Red | Excellent |
| 2 | Red/Pink | Good |
| 3 | Pink | Fair |
| 4 | Pink/White | Poor |
| 5 | White | Very poor |

(Van Wyk, 2002)

### 4.2.4 Statistical analyses

The PROC GLM procedure of SAS (2010) was used to analyse data for haematological indices (PCV, MCV, MCH, MCHC, RBC, Hb, WBC, lymphocytes and eosinophils). The PROC MIXED procedure of SAS (2010) was used to analyse for FAMACHA data. Comparison of least square means was done using the PDIFF option of SAS (2010). The model was used:

$Y_{ijkl} = \mu + \alpha_i + \beta_j + \lambda_k + \delta_l + (\alpha \times \beta)_{ij} + (\alpha \times \lambda)_{ik} + (\beta \times \lambda)_{jk} + (\alpha \times \beta \times \lambda)_{ijk} + \varepsilon_{ijkl}$;

Where; $Y_{ijkl}$ = response variable (FAMACHA);

$\mu$ = population mean;

$\alpha_i$ = dietary protein supplementation;

$\beta_j$ = dietary vitamin E supplementation;

$\lambda_k$ = week;

$\delta_l$ = sex;

$(\alpha \times \beta)_{ij}$ = interaction between dietary protein and vitamin E supplementation;

$(\alpha \times \lambda)_{ik}$ = interaction between dietary protein and week;

$(\beta \times \lambda)_{jk}$ = interaction between dietary vitamin E and week;

$(\alpha \times \beta \times \lambda)_{ijk}$ = interaction between protein, vitamin E and week;

$\varepsilon_{ijkl}$ = standard error.

The model used for haematological indices:

$Y_{ijk} = \mu + \alpha_i + \beta_j + \lambda_k (\alpha \times \beta)_{ij} + \varepsilon_{ijk}$;

$Y_{ijk}$ = response variable (PCV, WBC, RBC, Hb, MCV, MCH, MCHC, lymphocytes and eosinophils)

$\mu$ = population mean;

$\alpha_i$ = dietary protein supplementation;

$\beta_j$ = dietary vitamin E supplementation;

$\lambda_k$ = sex;

$(\alpha \times \beta)_{ij}$ = interaction of dietary protein and vitamin E supplementation;

$\varepsilon_{ijk}$ = standard error.

## 4.3 Results

### 4.3.1 FAMACHA

Dietary protein supplementation affected FAMACHA scores. Sex, week and dietary vitamin E supplementation did not affect FAMACHA scores. There was no interaction of dietary protein and week, and dietary vitamin E and week, and dietary protein, vitamin E and week on FAMACHA scores. There was an interaction ($P<0.05$) between dietary protein and vitamin E supplementation on FAMACHA scores (Table 4.2). As shown in Table 4.3, lambs that received the PE and PNE treatment had lower ($P<0.05$) FAMACHA scores, than lambs that received the NPE and NPNE treatment, which had higher ($P<0.05$) FAMACHA scores.

### 4.3.2 Packed cell volume and haemoglobin

There was an effect of dietary protein supplementation on PCV and Hb. Sex and dietary vitamin E supplementation did not affect PCV and Hb. There was no interaction ($P>0.05$) between dietary protein and vitamin E supplementation on PCV and Hb (Table 4.2).

### 4.3.3 Erythrocytes, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration

Dietary protein and vitamin E supplementation did not affect MCV (Table 4.2). Lambs that received the PE and NPE treatment had the same ($P>0.05$) MCV, whereas lambs that received PNE and NPNE treatment had lower ($P<0.05$) MCV (Table 4.4). Dietary protein and vitamin E supplementation affected MCHC and MCH (Table 4.2). Lambs that received the PE and PNE had higher ($P<0.05$) MCHC, than lambs that received NPE and NPNE treatment, which had the same ($P>0.05$) MCHC (Table 4.4). Dietary vitamin E supplementation did not affect RBC. Dietary protein supplementation did not affect RBC. There was no effect of sex on RBC, MCV, MCH and MCHC. There was no interaction between dietary protein and vitamin E supplementation on RBC, MCV, MCH and MCHC (Table 4.2).

### 4.3.4 Leucocytes, lymphocytes and eosinophils

Dietary protein and vitamin E supplementation did not affect WBC. Dietary vitamin E supplementation affected lymphocytes. Dietary protein supplementation did not affect lymphocytes. There was no interaction ($P>0.05$) between dietary protein and vitamin E supplementation on WBC and lymphocytes. Dietary protein and vitamin E supplementation had an effect on eosinophils. There was an interaction ($P<0.05$) between dietary protein and vitamin E supplementation on eosinophils (Table 4.2). As shown in Table 4.5, lambs that

received the PE and NPE treatment had the same ($P>0.05$) eosinophil concentration rather than lambs that received the PNE and NPNE treatment, which had higher ($P<0.05$) eosinophil concentrations. The sex of lambs was not significant on WBC, lymphocytes and eosinophil concentrations (Table 4.2).

**Table 4. 2: Level of significance for FAMACHA scores, packed cell volume (PCV), haemoglobin (Hb), erythrocytes (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin count (MCHC), leucocytes (WBC), lymphocytes and eosinophil concentration in lambs**

| **Effects** | **Traits** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **FAMACHA** | **PCV** | **Hb** | **RBC** | **MCHC** | **MCV** | **MCH** | **WBC** | **Lymphocytes** | **Eosinophils** |
| Protein | *** | * | * | * | * | NS | NS | NS | NS | * |
| Vitamin E | NS | NS | NS | NS | * | NS | * | NS | * | * |
| Protein × vitamin E | * | NS | NS | NS | NS | NS | NS | NS | NS | * |
| Sex | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Week | NS | - | - | - | - | - | - | - | - | - |
| Protein × week | * | - | - | - | - | - | - | - | - | - |
| Vitamin E × week | * | - | - | - | - | - | - | - | - | - |
| Protein × vitamin E × week | NS | - | - | - | - | - | - | - | - | - |

***P<0.001, *P<0.05, NS: Not significant (P>0.05), - : Not determined because blood samples were only collected at the end of the trial

**Table 4. 3: Least square means (± standard error) for interaction between dietary protein and vitamin E supplementation packed cell volume, haemoglobin and FAMACHA scores in lambs**

| | | PCV | |
|---|---|---|---|
| **Supplements** | | **E** | **NE** |
| | **P** | $0.42 \pm 0.03^{b}$ | $0.37 \pm 0.02^{bc}$ |
| | **NP** | $0.29 \pm 0.03^{ac}$ | $0.27 \pm 0.03^{a}$ |
| | | **Hb** | |
| | | **E** | **NE** |
| | **P** | $10.75 \pm 0.58^{b}$ | $10.22 \pm 0.52^{b}$ |
| | **NP** | $8.07 \pm 0.58^{a}$ | $9.90 \pm 0.67^{ab}$ |
| | | **FAMACHA** | |
| | | **E** | **NE** |
| | **P** | $1.40 \pm 0.10^{a}$ | $1.70 \pm 0.09^{b}$ |
| | **NP** | $2.75 \pm 0.10^{d}$ | $2.37 \pm 0.12^{c}$ |

[abcd] Values with different superscripts differ significantly ($P<0.05$).

P: protein; NP: no protein; E: vitamin E; NE: no vitamin E.

PCV: packed cell volume; Hb: haemoglobin

**Table 4. 4: Least square means (± standard error) for interaction between dietary protein and vitamin E supplementation erythrocytes, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration in lambs**

| | | RBC | |
|---|---|---|---|
| **Supplements** | | **E** | **NE** |
| | **P** | 9.47 ± 0.66[b] | 8.49 ± 0.59[ab] |
| | **NP** | 7.12 ± 0.66[a] | 6.95 ± 0.76[a] |
| | | **MCV** | |
| | | **E** | **NE** |
| | **P** | 40.77 ± 1.49[b] | 40.48 ± 1.34[b] |
| | **NP** | 40.70 ± 1.49[b] | 35.18 ± 1.73[a] |
| | | **MCH** | |
| | | **E** | **NE** |
| | **P** | 12.55 ± 0.29 | 11.80 ± 0.26 |
| | **NP** | 12.41 ± 0.29 | 11.75 ± 0.34 |
| | | **MCHC** | |
| | | **E** | **NE** |
| | **P** | 33.82 ± 0.49[b] | 32.40 ± 0.43[a] |
| | **NP** | 32.26 ± 0.49[a] | 31.22 ± 0.56[a] |

[ab] Values with different superscripts differ significantly (P<0.05).

P: protein; NP: no protein; E: vitamin E; NE: no vitamin E.

RBC: erythrocytes; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration

**Table 4. 5: Least square means for interaction between dietary protein and vitamin E supplementation on leucocytes, lymphocytes and eosinophils in lambs**

| | | WBC | |
|---|---|---|---|
| **Supplements** | | **E** | **NE** |
| | **P** | 8.02 ± 1.30 | 5.88 ± 1.16 |
| | **NP** | 6.65 ± 1.30 | 5.70 ± 1.50 |
| | | **Lymphocytes** | |
| | | **E** | **NE** |
| | **P** | 6.55 ± 0.61[b] | 5.44 ±0.54[ab] |
| | **NP** | 6.37 ± 0.61[ab] | 4.59 ± 0.70[a] |
| | | **Eosinophils** | |
| | | **E** | **NE** |
| | **P** | 0.18 ± 0.04[a] | 0.21 ± 0.04[a] |
| | **NP** | 0.20 ± 0.04[a] | 0.46 ± 0.05[b] |

[ab] Values with different superscripts differ significantly (P<0.05); WBC: leucocytes.

P: protein; NP: no protein; E: vitamin E; NE: no vitamin E

## 4.4 Discussion

The impact of gastrointestinal nematode on blood profile coupled with nematode resistance to anthelmintic is of great concern. Grazing susceptible lamb on heavily contaminated *Pennisetum clandestinum* increased lamb vulnerability to GIN infection. Nutritional potential of dietary protein and vitamin E supplementation has been explored. However, the interaction potential between dietary protein and vitamin E supplementation against parasitism is largely unknown. The need to supplement essential nutrients which are known to boost and improve the immune response cannot be overemphasized, since pasture or browses are in evocable and GIN are contacted under natural condition.

Dietary protein supplementation provides host body with essential amino acids lost when nematode sucks blood from the gut, which may reduce cell metabolic activities. The antioxidating potentials of dietary vitamin E are involved in suppressing free radical injuries caused by GIN in the gut (Smith and Bryant, 1989), and multiples T cells against foreign pathogens (Han, 2006). However, the success of dietary vitamin E supplementation to effectively combat foreign pathogens and increase cell immunity in an affected host may rely on sufficient amounts of dietary protein supplementation.

The normal ranges for haematological indices for sheep are WBC (4 – 8) × $10^9$/L, lymphocytes (2 – 9) × $10^9$/L, eosinophils (0 – 1) × $10^9$/L, RBC (9 – 15) × $10^9$/L, MCV (28 – 40) fL, MCH (8 – 12) pg/cell, MCHC (31 – 34) × 10 g/L, PCV (27 – 45) %, and Hb (9 – 15) g/dL (Jackson *et al*., 2002). The significance of dietary protein supplementation on FAMACHA scores shows that dietary protein supplementation may improve lambs health status. Dietary vitamin E supplementation had no influence on FAMACHA scores. The result on dietary vitamin E supplementation on FAMACHA scores may be due to the high parasite burden on *Pennisetum*

*clandestinum* and the inability of dietary vitamin E supplementation to work independently against parasitism. Its effectiveness on FAMACHA scores may depend on dietary protein supplementation. Their interaction shows that lambs maintained on a diet high in protein require sufficient amount of dietary vitamin E supplementation to effectively improve their health status (Martinez-Perez *et al*., 2014).

Dietary protein supplementation improved PCV in the present study agrees with Bricarello *et al*. (2004) who reported a significant effect of high dietary protein supplementation on PCV in lambs experimentally infected with a mixed infection of *Haemonchus* and *Trichostrongylus*. More so, supplementing dietary protein prevents GIN consequences and enhance lamb immunity against parasitism (Datta *et al*., 1999). The response of lambs on PCV in the present study confirmed that dietary protein supplementation increased PCV in lambs challenged with a mixture of GIN acquired under natural grazing pattern. The effect of dietary vitamin E supplementation on PCV in the present study agrees with MacGlafin *et al*. (2011), who reported that supplementing an injectable form of dietary vitamin E had no influence on PCV in lambs.

Lambs that received the PE treatment had an increased PCV compared to PNE, NPE and NPNE lambs. The response of the lambs in the PE treatment could be associated with the influence of dietary protein with dietary vitamin E supplementation in the maintenance of blood tissue (Martinez-Perez *et al*., 2014). The result on dietary protein supplementation on Hb shows that dietary protein may enhance cell and tissue activities and alter the damages caused by GIN manifestation which may reduce Hb concentration (Khan *et al*., 2012). The observation on Hb concentration in the present study agrees with Khan *et al*. (2012), who documented that dietary protein supplementation influenced Hb performance in experimentally infected lambs but

contrary to Louvadini *et al*. (2006), who reported a significant reduction in Hb concentrations in GIN infected lambs.

The result on dietary vitamin E and the interaction of dietary protein and vitamin E supplementation on Hb in lambs could be as a result of high worm burden as reported in the previous chapter. In addition, digestion and absorption of dietary vitamin E was probably slow, since the actual site of absorbing dietary vitamin E is the gut which is occupied by matured GIN (Larsen *et al*., 1988). However, lambs in the PE treatment had increased Hb, compared to the PNE, NPE and NPNE lambs.

The influence of dietary protein supplementation on RBC in the present study could be as a result of dietary protein function in preventing host tissue against intestinal haemorrhage caused by GIN (Simone *et al*., 2005). The observed result on the effect of dietary vitamin E supplementation on RBC agrees with Martinez-Perez *et al*. (2014), who reported no significant effect of dietary vitamin E supplementation on parasitized sheep. The result observed on the interaction of dietary protein and vitamin E supplementation was probably due to the mixed infection of *Haemonchus* and *Trichostrongylus* nematode species (Bordoloi *et al*., 2012) and the detrimental effect of GIN which causes anaemia and blood leakage from the attached site in the abomasa mucosa which may reduce RBC concentration in infected lambs (Sharma *et al*. 2000). Similarly, erythrocytes values were a little below the normal range for PNE, NPE and NPNE treatments lambs, however, PE treatment lambs performed better, as seen in Table 4.4.

Mean corpuscular volume explains the cause of anaemia in an infected animal (Rodriguez *et al*., 2015). However, MCV performance is influenced by RBC concentration in a host cell (Rodriguez *et al*., 2015). The result observed on dietary protein supplementation on MCV

could be associated to the low RBC volume in lambs (Rodriguez *et al*., 2015). Again, dietary vitamin E performance on MCV may be due to the low value observed for RBC concentration in treatment lambs. Interestingly, there was an influence of dietary protein with vitamin E supplementation on MCV. The current result on dietary protein and vitamin E supplementation on MCV shows a potential in improving lambs MCV performance in naturally infected lambs. Perhaps, a significant change might be observed if the quantity of dietary vitamin E supplementation was slightly increased for the treatment lambs.

Dietary protein supplementation influenced MCH performance in lambs and was similar to the response on MCV. The result observed on dietary protein supplementation on MCH may be due to the low value of RBC, since MCH is estimated as the average mass of haemoglobin per erythrocytes. The result on dietary vitamin E supplementation on MCH in parasitized lambs agrees with the early findings of Martinez-Perez *et al*. (2014) who argued that dietary vitamin E supplementation significantly improves MCH performance in parasited lambs. These results confirmed the potential of dietary vitamin E supplementation on intestinal parasite of sheep. Lambs in the PE treatment had a higher MCH value compared to the PNE, NPE and NPNE treatment lambs.

Mean corpuscular haemoglobin concentration is an associated RBC assessment which gives the average concentration of Hb. The response of dietary protein supplemented lambs on MCHC could be as a result of Hb performance in lambs which directly influence their response on MCHC. Documenting this result help understand how the influence of dietary protein supplementation can improve MCHC performance in GIN infected lambs. Similarly, the observation on dietary vitamin E supplementation again agrees with Martinez-Perez *et al*. (2014) who explained that dietary vitamin E supplementation even at 0.06 g/kg DM improved

MCHC performance of parasitized lambs. The values recorded for dietary vitamin E was not significantly different from dietary protein supplementation in the present study. Thus, dietary protein or vitamin E supplementation could be used to improve MCHC in parasitized lambs.

The results observed on the individual performance of dietary protein and vitamin E supplementation reflected on their interaction. The response by the supplemented lamb was probably due to the influence of dietary protein supplementation on dietary vitamin E supplemented lambs. Similarly, lambs in the PE treatment had higher MCHC compared to those in PNE, NPE and NPNE treatment. All treatment lambs maintained MCHC values within range.

Leucocytes are responsible for immune protection against infection and foreign pathogen, including GIN. The effect of dietary protein supplementation on WBC was expected since dietary protein supplementation improves the host immune function against foreign pathogens. The result on the effect of dietary protein supplementation on lymphocytes was suggested to be due to the mixed infection of nematode genera, *Haemonchus* and *Trichostrongylus*. The effect of dietary vitamin E supplementation in the present study agrees with Martinez-Perez *et al*. (2014), who reported that dietary vitamin E supplementation did not affect WBC in infected lambs. However, the sheep in the study show signs of leucocytosis due to an abnormal increase in WBC (Martinez-Perez *et al*. 2014). Lambs in the present study had WBC value within the normal range.

The result on dietary vitamin E supplementation on lymphocytes in the present study disagrees with Martinez-Perez *et al*. (2014) who observed no significant effect of dietary vitamin E supplementation on lymphocytes. The improved lymphocytes response in the current study

proved that dietary vitamin E supplementation could have triggered the release of antibodies or rather stimulates T helper cells which are required to strengthen the immune system against GIN manifestation (Han, 2006). The lymphocyte concentration in the current study was within the normal range for treatment lambs. Eosinophil concentration is usually relatively low, except in cases of allergies (Miller and Horohov, 2006). Therefore, a large value of eosinophil concentration could be as a result of parasitic activities in the intestinal compartment of the host, which may damage and cause inflammation or haemorrhage in the intestinal wall (Larsen *et al*., 1988; Miller and Horohov, 2006).

The significance of dietary protein on eosinophil concentration in the current study again agrees with Martinez-Perez *et al*. (2014), who observed a significant effect on eosinophil concentration when lambs are supplied a diet high in protein. Similarly, the effect of dietary vitamin E supplementation measured in the blood cells of lambs improved in the current study compared to De wolf *et al*. (2014), who measured eosinophil concentration in the abomasum of host and observed no significant response in treatment lambs eosinophil concentration.

There was an improvement in dietary protein supplemented lambs due to dietary vitamin E supplementation. The interaction of dietary protein and vitamin E supplementation could have prevented lambs against the inflammatory processes and haemorrhage caused by GIN through sloughing of the intestinal wall of an infected host (Valentine *et al*., 2007). Lambs in the PE treatment had fewer eosinophil concentration compared to the PNE, NPE and NPNE treatment lambs. Dietary protein and vitamin E supplementation may sustain lambs health status.

## 4.5 Conclusions

There was an interaction between dietary protein and vitamin E supplementation on eosinophil concentration, MCV, MCHC and FAMACHA in lambs. Hence, the immune response of dietary protein supplemented lamb on eosinophil concentration, MCV, MCHC and FAMACHA performance is influenced by dietary vitamin E supplementation. However, elucidating the interaction of dietary protein and vitamin E supplementation on biochemical performance and immunoglobulins is warranted.

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# CHAPTER 5

## General Discussion, Conclusions and Recommendations

### 5.1 General discussion

Gastrointestinal nematodes deteriorating consequences led to the review of nematode measures and control strategies. Nematode resistance to anthelmintics and natural grazing management are constraints to lamb productivity and health sustainability. Poor nutritional status of host increases lamb vulnerability, and support GIN pathophysiological growth and consequences. Gastrointestinal parasites alter the digestion and absorption of feed stuff into the gut for growth, and cell maintenance and integrity. Intestinal parasites affect dietary protein synthesis in infected host. Dietary vitamin E supplementation enhance efficient maintenance and function of the intestinal tissue and haematological responses. It was observed that through the potential of dietary protein and vitamin E supplementation, lamb performance may be improved through the interaction of dietary protein and vitamin E supplementation.

Chapter 3 of the present study assessed the interaction of dietary protein and vitamin E supplementation on faecal egg count, cultured *Haemonchus* and *Trichostrongylus* larvae, average daily gains and body condition scores in lambs. The hypothesis tested was that the interaction of dietary protein and vitamin E supplementation will reduce FEC, $CHL_3$ and $CTL_3$, and increase ADG and BCS in GIN naturally infected lambs. The interaction of dietary protein and vitamin E supplementation reduced FEC, $CHL_3$ and $CTL_3$. It was demonstrated that the interaction of dietary protein and vitamin E supplementation subsided GIN pathophysiological consequences in naturally infected lambs. The hypothesis was, therefore, not rejected.

To further enhance lamb performance, while minimising the risks associated with GIN consequences. Chapter 4 investigated the interaction between dietary protein and vitamin E supplementation on haematological response and FAMACHA scores. It was hypothesized that, to achieve a responsive haematological performance and FAMACHA scores, lambs fed dietary protein supplementation may require dietary vitamin E supplementation. The interaction between dietary protein and vitamin E supplementation enhanced lower eosinophil production and FAMACHA scores. Similarly, an increase in mean corpuscular volume and mean corpuscular haemoglobin concentration was also demonstrated. The interaction between dietary protein and vitamin E supplementation improved lambs health status. Therefore, the hypothesis tested was not rejected.

## 5.2 Conclusions

The interaction between dietary protein (150 g) and vitamin E (30 IU/kg BW/day) supplementation reduced faecal egg counts, *Haemonchus* and *Trichostrongylus* larvae. In addition, the interaction of dietary protein and vitamin E supplementation improved eosinophil concentrations, mean corpuscular volume, and mean corpuscular haemoglobin concentration and FAMACHA scores. Dietary protein and vitamin E supplementation enhanced lambs health status. Hence, the effective responsiveness of dietary protein supplemented lambs depend on dietary vitamin E supplementation. Therefore, nutritional therapy has the potential of controlling parasitism and maybe sustainable against nematodiosis.

## 5.3 Recommendations

It is recommended that farmers may feed dietary protein (150 g/day) and vitamin E (30 IU/kg BW/day) supplementation in the control of parasitism, especially during the dry season when nutrient quality and quantity diminishes, and climatic condition favours the morphological

growth and survival of gastrointestinal nematodes. Supplementing, dietary protein and vitamin E will enhance productivity and reduce the risk associated with nematodiosis.

Further research recommendations are:

- To investigate the interaction of dietary protein and vitamin E supplementation on biochemical response in naturally infected lambs.
- To elucidate the interaction of dietary protein and vitamin E supplementation on the growth of adult male and female worm survival in the abomasa mucosa of lambs.
- To assess the interaction of dietary protein and vitamin E supplementation on histopathological response in lambs. Understanding the production of antibodies at the site of infection will help establish the length at which nutrition can avert nematode damages in the gastrointestinal tract and tissue.
- To determine the influence of dietary protein and vitamin E supplementation in improving immunoglobulin production against nematodiosis.

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

12 October 2015

**Mr Ayobami Adeyemo**
**School of Agricultural, Earth & Environmental Sciences**
**Westivlle Campus**

Dear Mr Adeyemo

**Protocol reference number: AREC/096/015M**
**Project title:** Interaction of protein and vitamin E supplementation on lamb growth, parasite load and serum chemistry

**Full Approval – Research Application**

With regards to your application received on 27 August 2015. The documents submitted have been accepted by the Animal Research Ethics Committee and **FULL APPROVAL** for the protocol has been granted with following conditions.

**CONDITIONS:**

1. **Animals must be monitored daily - at least once.**
2. **If any adverse symptoms arise due to the change of diet, please inform the Animals Research Ethics Committee immediately.**

**Any alteration/s to the approved research protocol, i.e Title of Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number.**

Please note: Research data should be securely stored in the discipline/department for a period of 5 years.

**The ethical clearance certificate is only valid for a period of one year from the date of issue. Renewal for the study must be applied for before 12 October 2016.**

I take this opportunity of wishing you everything of the best with your study.

Yours faithfully

.....

**Dr S Islam**
**Chair: Animal Research Ethics Committee**

**/ms**

Cc Superivsor: Dr M Chimonyo
Cc Dean & Head of School: Prof A Modi
Cc Acting Registrar: Professor D Jaganyi
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## Appendix 1: University of KwaZulu-Natal Animal Ethics Research Committee Clearance (Reference number: AREC/096/015M)